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(71) Applicant *(for all designated States except US)*: BOEHRINGER INGELHEIM (CANADA) LTD. [CA/CA]; 2100 Cunard, Laval, Québec H7S 2G5 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LLINAS–BRUNET, Montse [CA/CA]; 10543 Bélair, Pierrefonds, Québec H2V 2W8 (CA). BAILEY, Murray, Douglas [CA/CA]; 344 Groulx, Pierrefonds, Québec H8Y 1B3 (CA). HALMOS, Teddy [CA/CA]; 1935 Jean Ricard #8, Laval, Québec H7T 2K4 (CA). POUPART, Marc–André [CA/CA]; 101 Aimé Séguin, Laval, Vimont, Québec H7M 1B3 (CA). TSANTRIZOS, Youla [–/–]; 1590 Champigny, Saint–Laurent, Québec H4L 4P7 (CA).

(74) Agent: VAN ZANT, Joan, M.; Van Zant & Associates, Suite 1407, 77 Bloor Street West, Toronto, Ontario M5S 1M2 (CA).

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(54) Title: HEPATITIS C INHIBITOR PEPTIDE ANALOGUES

(57) Abstract

Compound of formula (I) active against the Hepatitis C virus, wherein B is an acyl derivative; a is 0 or 1; R_6, when present, is carboxy(lower)alkyl; b is 0 or 1; R_5, when present, is C_{1-6} alkyl, or carboxy(lower)alkyl; Y is H or C_{1-6} alkyl; R_4 is C_{1-10} alkyl; R_3 is C_{1-10} alkyl; W is $-NH-CH(R_2)-C(O)-$, wherein R_2 is C_{1-6} alkyl; C_6 or C_{10} aryl; C_{7-16} aralkyl; or carboxy(lower)alkyl; or W is a proline derivative; Q is a group of the formula $-Z(R_1)-C(O)-R_{13}$, wherein Z is CH or N; R_1 is C_{1-6} alkyl or C_{1-6} alkenyl both optionally substituted with thio or halo; and R_{13} is an activated carbonyl substituent, or Q is a phosphonate group of the formula $-CH(R_1)-P(O)R_{15}R_{16}$ wherein R_{15} and R_{16} are independently C_{6-20} aryloxy; and R_1 is as defined above.

Hepatitis C Inhibitor Peptide Analogues

Field of the invention

5   The present invention relates to compounds,
     compositions and methods for the treatment of
     hepatitis C virus (HCV) infection. In particular,
     the present invention provides novel peptides and
     analogues thereof, pharmaceutical compositions
10   containing such peptides and methods for using these
     peptides in the treatment of HCV infection.

Background of the invention

15   Hepatitis C virus (HCV) is the major etiological
     agent of post-transfusion and community-acquired non-
     A non-B hepatitis worldwide. It is estimated that
     over 100 million people worldwide are infected by the
     virus. A high percentage of carriers become
20   chronically infected and many progress to chronic
     liver disease, so called chronic hepatitis C. This
     group is in turn at high risk for serious liver
     disease such as liver cirrhosis, hepatocellular
     carcinoma and terminal liver disease leading to
25   death.

     The mechanism by which HCV establishes viral
     persistence and causes a high rate of chronic liver
     disease has not been thoroughly elucidated. It is
30   not known how HCV interacts with and evades the host
     immune system. In addition, the roles of cellular
     and humoral immune responses in protection against
     HCV infection and disease have yet to be established.
     Immunoglobulins have been reported for prophylaxis of

transfusion-associated viral hepatitis. However, the
Center for Disease Control does not presently
recommend immunoglobulins for this purpose.

5   The lack of an effective protective immune response
      is hampering the development of a vaccine or adequate
      post-exposure prophylaxis measures, so in the near-
      term, hopes are firmly pinned on antiviral
      interventions.

10

      Various clinical studies have been conducted with the
      goal of identifying pharmaceutical agents capable of
      effectively treating HCV infection in patients
      afflicted with chronic hepatitis C. These studies

15   have involved the use of interferon-alpha, alone and
      in combination with other antiviral agents. Such
      studies have shown that a substantial number of the
      participants do not respond to these therapies, and
      of those that do respond favorably, a large

20   proportion were found to relapse after termination of
      treatment.

      Until recently, interferon (IFN) was the only
      available therapy of proven benefit approved in the

25   clinic for patients with chronic hepatitis C. However
      the sustained response rate is low, and interferon
      treatment also induces severe side-effects (i.e.
      retinopathy, thyroiditis, acute pancreatitis,
      depression) that diminish the quality of life of

30   treated patients. Recently, interferon in
      combination with ribavirin has been approved for
      patients non-responsive to IFN alone. However, the
      side effects caused by IFN are not alleviated with
      this combination therapy.

Therefore, a need exists for the development of
effective antiviral agents for treatment of HCV
5   infection that overcomes the limitations of existing
pharmaceutical therapies.

HCV is an enveloped positive strand RNA virus in the
Flaviviridae family. The single strand HCV RNA genome
10   is approximately 9500 nucleotides in length and has a
single open reading frame (ORF) encoding a single
large polyprotein of about 3000 amino acids. In
infected cells, this polyprotein is cleaved at
multiple sites by cellular and viral proteases to
15   produce the structural and non-structural (NS)
proteins. In the case of HCV, the generation of
mature nonstructural proteins (NS2, NS3, NS4A, NS4B,
NS5A, and NS5B) is effected by two viral proteases.
The first one, as yet poorly characterized, cleaves
20   at the NS2-NS3 junction; the second one is a serine
protease contained within the N-terminal region of
NS3 (henceforth referred to as NS3 protease) and
mediates all the subsequent cleavages downstream of
NS3, both in *cis*, at the NS3-NS4A cleavage site, and
25   in *trans*, for the remaining NS4A-NS4B, NS4B-NS5A,
NS5A-NS5B sites. The NS4A protein appears to serve
multiple functions, acting as a cofactor for the NS3
protease and possibly assisting in the membrane
localization of NS3 and other viral replicase
30   components. The complex formation of the NS3 protein
with NS4A seems necessary to the processing events,
enhancing the proteolytic efficiency at all of the
sites. The NS3 protein also exhibits nucleoside
triphosphatase and RNA helicase activities. NS5B is

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a RNA-dependent RNA polymerase that is involved in the replication of HCV.

A general strategy for the development of antiviral agents is to inactivate virally encoded enzymes that are essential for the replication of the virus. In this vein, patent application WO 97/06804 describes the (-) enantiomer of the nucleoside analogue cytosine-1,3-oxathiolane (also known as 3TC) as active against HCV. This compound, although reported as safe in previous clinical trials against HIV and HBV, has yet to be clinically proven active against HCV and its mechanism of action against the virus has yet to be reported.

Intense efforts to discover compounds which inhibit the NS3 protease or RNA helicase of HCV have led to the following disclosures:

- US patent 5,633,388 describes heterocyclic-substituted carboxamides and analogues as being active against HCV. These compounds are directed against the helicase activity of the NS3 protein of the virus but clinical tests have not yet been reported.

- A phenanthrenequinone has been reported by Chu *et al* (Tet. Lett., (1996), 7229-7232) to have activity against the HCV NS3 protease *in vitro*. No further development on this compound has been reported.

- A paper presented at the Ninth International Conference on Antiviral Research, Urabandai, Fukyshima, Japan (1996) (Antiviral Research, <u>30</u>,

5

1, 1996; A23 (abstract 19)) reports thiazolidine
derivatives to be inhibitory to the HCV protease.

Several studies have reported compounds inhibitory to
5   other serine proteases, such as human leukocyte
elastase. One family of these compounds is reported
in WO 95/33764 (Hoechst Marion Roussel, 1995). The
peptides disclosed in that application are
morpholinylcarbonyl-benzoyl-peptide analogues that
10   are structurally different from the peptides of the
present invention.

- WO 98/17679 from Vertex Pharmaceuticals Inc.
  discloses inhibitors of serine protease,
15   particularly, Hepatitis C virus NS3 protease.
  These inhibitors are peptide analogues based on
  the NS5A/5B natural substrate that contain C-
  terminal aldehydes, α-ketoamides and fluorinated
  ketones.

20   - Hoffman LaRoche has also reported hexapeptides
  that are proteinase inhibitors useful as antiviral
  agents for the treatment of HCV infection. These
  peptides contain an aldehyde or a boronic acid at
  the C-terminus.

25   - Steinkühler *et al*. and Ingallinella *et al*. have
  published on NS4A-4B product inhibition
  (Biochemistry (1998), <u>37</u>, 8899-8905 and 8906-
  8914). However, these peptides and analogues were
  published after the priority date of the present
30   application.

One advantage of the present invention is that it
provides peptides that are inhibitory to the NS3
protease of the hepatitis C virus.

Summary of the invention

We investigated peptides potentially inhibitory to the NS3 protease. The discovery that the N-terminal cleavage product **(Ac-D-D-I-V-P-C-OH)** of an analogue of a natural substrate of the NS3 protease was inhibitory led us to the peptide analogues of the invention.

Included in the scope of the invention are compounds of formula (I):

(I)
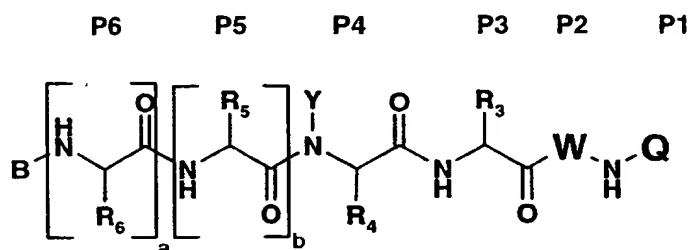
wherein **B** is an acyl derivative of formula R_{11}-C(O)- wherein R_{11} is C_{1-10} alkyl C_{3-10} cycloalkyl optionally substituted with carboxyl; or R_{11} is C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with a C_{1-6} alkyl; **a** is 0 or 1;

R_6, when present, is carboxy(lower)alkyl;

b is 0 or 1;

R_5, when present, is C_{1-6} alkyl, or carboxy(lower)alkyl;

Y is H or C_{1-6} alkyl;

R_4 is C_{1-10} alkyl; cycloalkyl C_{3-10};

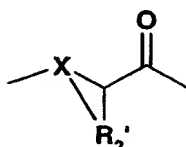R_3 is C_{1-10} alkyl; cycloalkyl C_{3-10};

W is a group of formula II:

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Formula II

wherein R_2 is C_{1-10} alkyl or C_{3-7} cycloalkyl optionally substituted with carboxyl; C_6 or C_{10} aryl; or C_{7-16} aralkyl; or

W is a group of formula II':

Formula II'

wherein **X** is CH or N; and

R_2' is C_{3-4} alkylene that joins **X** to form a 5- or 6- membered ring, said ring optionally substituted with OH; SH; NH_2; carboxyl; R_{12}; OR_{12}, SR_{12}, NHR_{12} or $NR_{12}R_{12}'$ wherein R_{12} and R_{12}' are independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl, said alkyl or alkenyl optionally substituted with NH_2, OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N; or

R_{12} and R_{12}' are independently C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} alkyl, NH_2, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

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said cyclic alkyl, cyclic alkenyl, aryl or
aralkyl being optionally fused with a second 5-
, 6- or 7-membered ring to form a cyclic system
or heterocycle, said second ring being
optionally substituted with NH_2, OH, SH, halo,
carboxyl or carboxy(lower)alkyl; said second
ring optionally containing at least one
heteroatom selected independently from the group
consisting of: O, S, and N;

Q is a group of the formula:

wherein **Z** is CH or N;

X is O or S;

R₁ is H, C_{1-6} alkyl or C_{1-6} alkenyl both optionally
substituted with thio or halo;
and

when **Z** is CH, then **R₁₃** is H; CF_3; CF_2CF_3; CH_2-**R₁₄**;
$CH(F)$-**R₁₄**; CF_2-**R₁₄**; $NR_{14}R_{14}'$ S-**R₁₄**; or CO-NH-**R₁₄** wherein
R₁₄ and **R₁₄'** are independently hydrogen, cyclic
C_{3-10} alkyl or acyclic C_{1-10} alkyl or cyclic C_{3-10}
alkenyl or acyclic C_{2-10} alkenyl, said alkyl or
alkenyl optionally substituted with NH_2, OH, SH,
halo or carboxyl; said alkyl or alkenyl
optionally containing at least one heteroatom
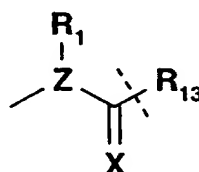selected independently from the group consisting
of: O, S, and N; or
R₁₄ and **R₁₄'** are independently C_6 or C_{10} aryl or
C_{7-16} aralkyl optionally substituted with C_{1-6}
alkyl, NH_2, OH, SH, halo, carboxyl or
carboxy(lower)alkyl or substituted with a
further C_{3-7} cycloalkyl, C_6 or C_{10} aryl, or

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heterocycle; said aryl or aralkyl optionally
containing at least one heteroatom selected
independently from the group consisting of: O,
S, and N;

5   said cyclic alkyl, cyclic alkenyl, aryl or
aralkyl being optionally fused with a second 5-,
6-, or 7-membered ring to form a cyclic system
or heterocycle, said second ring being
optionally substituted with NH_2, OH, SH, halo,

10   carboxyl or carboxy(lower)alkyl or substituted
with a further C_{3-7} cycloalkyl, C_6 or C_{10} aryl, or
heterocycle; said second ring optionally
containing at least one heteroatom selected
independently from the group consisting of: O,

15   S, and N;
or R_{14} and $R_{14'}$ are independently C_{1-4} alkyl which
when joined together with N form a 3 to 6-
membered nitrogen-containing ring which is
optionally fused with a further C_{3-7} cycloalkyl,

20   C_6 or C_{10} aryl or heterocycle;
with the proviso that when Z is CH, then R_{13} is not
an α-amino acid or an ester thereof;

when Z is N, then R_{13} is H; carboxy; C_{1-6} alkyl
25   optionally substituted with carboxy; CH_2-R_{14};
$CHR_{14}R_{14'}$; $CH(F)$-R_{14}; O-R_{14}; $NR_{14}R_{14'}$ or S-R_{14} wherein R_{14}
and $R_{14'}$ are as defined above; or

Q is a phosphonate group of the formula:

$$\underset{\overset{\|}{O}}{P}\overset{R_1}{\underset{R_{16}}{\diagdown}}R_{15}$$
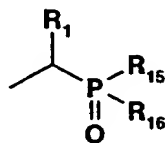
30

wherein R_{15} and R_{16} are independently C_{6-20} aryloxy; and R_1 is as defined above.

5   Included within the scope of this invention is a pharmaceutical composition comprising an anti-hepatitis C virally effective amount of a compound of formula I, or a therapeutically acceptable salt or ester thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.

10

An important aspect of the invention involves a method of treating a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of the compound
15   of formula I, or a therapeutically acceptable salt or ester thereof or a composition as described above.

Another important aspect involves a method of inhibiting the replication of hepatitis C virus by
20   exposing the virus to a hepatitis C viral NS3 protease inhibiting amount of the compound of formula I, or a therapeutically acceptable salt or ester thereof or a composition as described above.

25   Still another aspect involves a method of treating a hepatitis C viral infection in a mammal by administering thereto an anti-hepatitis C virally effective amount of a combination of the compound of formula I, or a therapeutically acceptable salt or
30   ester thereof, and an interferon. A pharmaceutical composition comprising the combination in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent is also within the scope of this invention.
35

11
Detailed description of the invention

As used herein, the following definitions apply
unless otherwise noted:

5

With reference to the instances where (R) or (S) is
used to designate the configuration of a radical,
e.g. R_4 of the compound of formula I, the designation
is done in the context of the compound and not in the
10   context of the radical alone.

The natural amino acids, with exception of glycine,
contain a chiral carbon atom. Unless otherwise
specifically indicated, the compounds containing
15   natural amino acids with the L-configuration are
preferred. However, applicants contemplate that when
specified, some amino acids of the formula I can be
of either D- or L- configuration or can be mixtures
of D- and L-isomers, including racemic mixtures.

20

The designation "P1, P2, P3 et." as used herein refer
to the position of the amino acid residues starting
from the C-terminus end of the peptide analogues and
extending towards the N-terminus (i.e. P1 refers to
25   position 1 from the C-terminus, P2: second position
from the C-terminus, etc.) (see Berger A. & Schechter
I., Transactions of the Royal Society London series,
(1970), B257, 249-264).

30   The abbreviations for the α-amino acids are set forth
in Table A.

12

Table A

AMINO ACID	SYMBOL
Allylglycine	AlGly
Aminobutyric acid	Abu
Alanine	Ala
Aspartic acid	Asp
Cysteine	Cys
Cyclohexylalanine	Cha
Cyclohexylglycine (also named: 2-amino-2-cyclohexylacetic acid)	Chg
Glutamic acid	Glu
Isoleucine	Ile
Leucine	Leu
Norvaline	Nva
Phenylalanine	Phe
Pipecolic acid	Pip
Proline	Pro
4(R)-Hydroxyproline	Hyp
4(R)-Benzyloxyproline	Hyp(4-Bn)
Valine	Val
tert-Butylglycine	Tbg

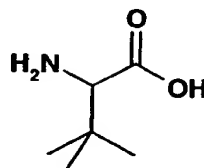As used herein the term "aminobutyric acid" refers to a compound of formula:
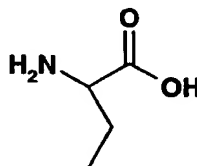
5

As used herein the term "allylglycine" refers to a compound of formula:

13

$$H_2N \overset{\displaystyle O}{\underset{\displaystyle}{\|}} OH$$
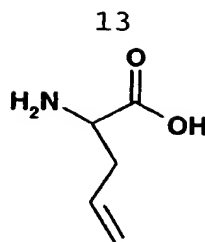
As used herein the term "*tert*-butylglycine" refers to a compound of formula:

5

The term "residue" with reference to an amino acid or amino acid derivative means a radical derived from the corresponding α-amino acid by eliminating the

10 hydroxyl of the carboxy group and one hydrogen of the α-amino group. For instance, the terms Gln, Ala, Gly, Ile, Arg, Asp, Phe, Ser, Leu, Cys, Asn, Sar and Tyr represent the "residues" of L-glutamine, L-alanine, glycine, L-isoleucine, L-arginine, L-aspartic acid,

15 L-phenylalanine, L-serine, L-leucine, L-cysteine, L-asparagine, sarcosine and L-tyrosine, respectively.

The term "side chain" with reference to an amino acid or amino acid residue means a group attached to the

20 α-carbon atom of the α-amino acid. For example, the R-group side chain for glycine is hydrogen, for alanine it is methyl, for valine it is isopropyl. For the specific R-groups or side chains of the α-amino acids reference is made to A.L. Lehninger's

25 text on Biochemistry (see chapter 4).

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The term "halo" as used herein means a halogen radical selected from bromo, chloro, fluoro or iodo.

The term "C_{1-6} alkyl" or "(lower)alkyl" as used
5   herein, either alone or in combination with another radical, means straight chain or branched alkyl radicals containing up to six carbon atoms and includes, for example, methyl, ethyl, propyl, butyl, hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl,
10   1,1-dimethylethyl.

Likewise, the terms "C_{1-3} alkyl" "C_{1-4} alkyl" and "C_{1-10} alkyl" are used to denote alkyl radials containing up to three, four and ten carbon atoms, respectively.
15

The term "C_{3-7} cycloalkyl" as used herein, either alone or in combination with another radical, means a cycloalkyl radical containing from three to seven carbon atoms and includes cyclopropyl, cyclobutyl,
20   cyclopentyl, cyclohexyl and cycloheptyl.

The term "C_{4-10} (alkylcycloalkyl) as used herein means a cycloalkyl radical containing from three to seven carbon atoms linked to an alkyl radical, the linked
25   radicals containing up to ten carbon atoms; for example, cyclopropylmethyl, cyclopentylethyl, cyclohexylmethyl, cyclohexylethyl or cycloheptyl-ethyl.

30   The term "C_{2-10} alkenyl" as used herein, either alone or in combination with another radical, means an alkyl radical as defined above containing from 2 to 10 carbon atoms, and further containing at least one double bond. For example alkenyl includes allyl.

The term "C_{3-4} alkylene" as used herein means a
divalent alkyl radical derived by the removal of two
hydrogen atoms from a straight or branched chain
5   aliphatic hydrocarbon containing from three to four
carbon atoms and includes, for example, $-CH_2CH_2CH_2-$,
$CH(CH_3)CH_2CH_2-$, $-CH_2C(CH_3)_2-$ and $-CH_2CH_2CH_2CH_2-$.

The term "C_{1-6} alkoxy" as used herein, either alone or
10   in combination with another radical, means the
radical $-O-C_{1-6}$ alkyl wherein alkyl is as defined
above containing up to six carbon atoms. Alkoxy
includes methoxy, ethoxy, propoxy, 1-methylethoxy,
butoxy and 1,1-dimethylethoxy. The latter radical is
15   known commonly as *tert*-butoxy.

The term "C_6 or C_{10} aryl" as used herein, either alone
or in combination with another radical, means either
an aromatic monocyclic system containing 6 carbon
20   atoms or an aromatic cyclic system containing 10
carbon atoms.

The term "C_{7-16} aralkyl" as used herein, either alone
or in combination with another radical, means an aryl
25   as defined above linked through an alkyl group,
wherein alkyl is as defined above containing from 1
to 6 carbon atoms. Aralkyl includes for example
benzyl, and butylphenyl.

30   The term "carboxy(lower)alkyl" as used herein, either
alone or in combination with another radical, means a
carboxyl group (COOH) linked through a (lower)alkyl
group as defined above and includes for example
butyric acid or the groups:

or
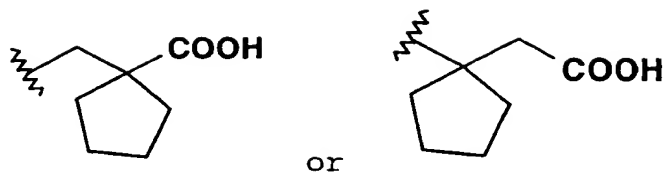
The term "cyclic" or "cyclic system" as used herein,
either alone or in combination with another radical,
means a monovalent radical derived by removal of a
hydrogen from a saturated or unsaturated cyclic
hydrocarbon, containing from three to seven carbon
atoms, unless otherwise indicated and optioonally
conctaing one or more heteroatom. The term cyclic or
cyclic system includes, for example, cyclopropane,
cyclopentane, cyclohexane, cyclohexene, decalin,
tetralin, indene, and naphthalene.

The term "heterocycle" as used herein, either alone
or in combination with another radical, means a
monovalent radical derived by removal of a hydrogen
from a five-, six-, or seven-membered saturated or
unsaturated heterocycle containing from one to four
heteroatoms selected from nitrogen, oxygen and
sulfur. Examples of suitable heterocycles include:
pyrrolidine, tetrahydrofuran, thiazolidine, pyrrole,
thiophene, diazepine, 1H-imidazole, 1-methyl-1H-
imidazole, isoxazole, thiazole, 2-methylthiazole, 2-
aminothiazole, piperidine, 1,4-dioxane, 4-morpholine,
pyridine, 2-methylpyridine, pyrimidine, 4-
methylpyrimidine and 2,4-dimethylpyrimidine.

The term "heterocyclic system" as used herein, either
alone or in combination with another radical, means a
heterocycle as defined above fused to one or more
other cycle be it a heterocycle or any other cycle.

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Examples of suitable heterocyclic systems include:
thiazolo[4,5-b]-pyridine, quinoline, or indole.

Preferred embodiments

5   A further preferred group of compounds are
represented by formula I wherein **B** is preferably an
acyl derivative of formula $R_{11}C(O)$- wherein R_{11} is:
C_{1-6} alkyl optionally substituted with carboxyl, C_{1-6}
alkanoyloxy or C_{1-6} alkoxy;

10   C_{3-7} cycloalkyl optionally substituted with carboxyl,
MeOC(O), EtOC(O) or BnOC(O);
3-carboxypropionyl (DAD) or 4-carboxybutyryl (DAE);
or
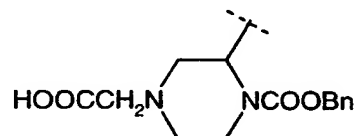
15   More preferably, **B** is acetyl, 3-carboxypropionyl, 4-
carboxylbutyryl, $AcOCH_2C(O)$, $Me_3COC(O)$,
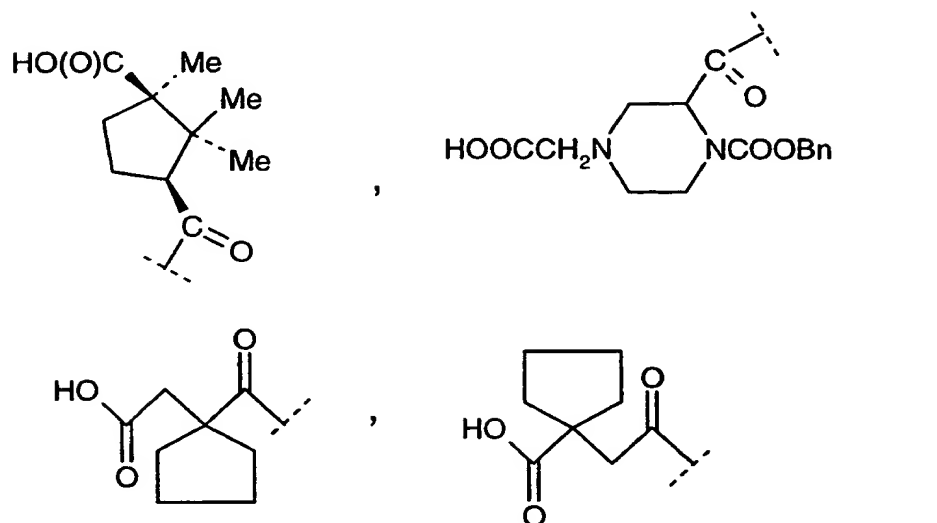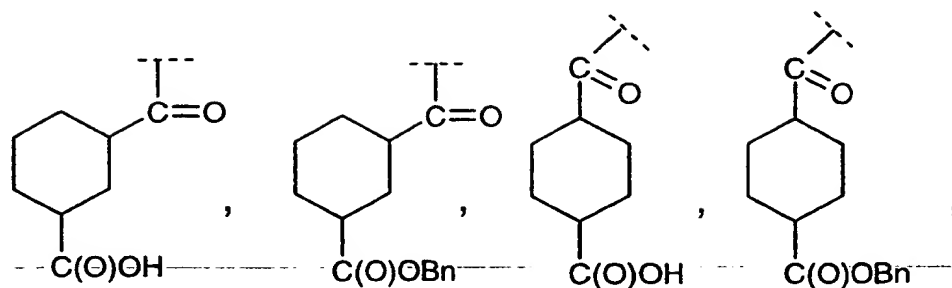
Still, more preferably, **B** is acetyl, 3-
carboxypropionyl (DAD), 4-carboxybutyryl (DAE),
5   AcOCH$_2$C(O),
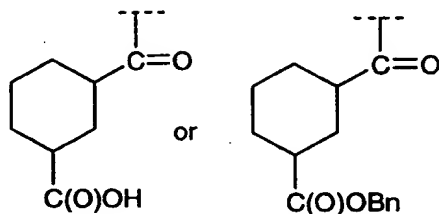
Most preferably, **B** is acetyl, or 4-carboxybutyryl
(DAE). Preferably, **B** is acetyl.

10   Preferably, **R$_6$,** when present, is the side chain of
Asp or Glu.
Most preferably, **R$_6$,** when present, is the side chain
of Asp.

19

Alternatively, preferably, **a** is 0 and then **R₆** is absent.

Preferably, **R₅**, when present, is the side chain of an amino acid selected from the group consisting of: D-Asp, L-Asp, D-Glu, L-Glu, D-Val, L-Val, D-*tert*-butylglycine (Tbg), and L-Tbg.

More preferably, **R₅**, when present, is the side chain of D-Asp, D-Val, or D-Glu.

Most preferably, **R₅**, when present, is the side chain of D-Glu.

Alternatively, preferably **a** is 0 and **b** is 0, and then both **R₆** and **R₅** are absent.

Preferably, **Y** is H or C₁₋₃ alkyl.

More preferably, **Y** is Me.

Alternatively, more preferably **Y** is H.

Preferably, **R₄** is the side chain of an amino acid selected from the group consisting of: Val, cyclohexylglycine (Chg), Tbg, Ile, or Leu.

More preferably, **R₄** is the side chain of Chg or Ile.
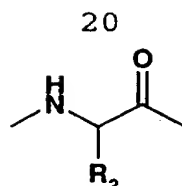
Most preferably, **R₄** is the side chain of Chg.

Preferably, **R₃** is the side chain of an amino acid selected from the group consisting of: Ile, Chg, Cha, Val or Glu.

More preferably, **R₃** is the side chain of Val or Chg.
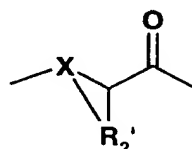
Most preferably, **R₃** is the side chain of Val.

Preferably, **W** is a group of formula II:

20

wherein R_2 is C_{1-8} alkyl or C_{3-6} cycloalkyl optionally substituted with carboxyl; or benzyl. More preferably, R_2 is the side chain of Asp, aminobutyric

5   acid (Abu) or Val.

Still, more preferably, **W** is a group of formula II':

10   wherein preferably, **X** is CH or N.

More preferably R_2' is a C_3 or C_4 alkylene (shown in black) that joins **X** to form a 5- or 6-membered ring of formula III:
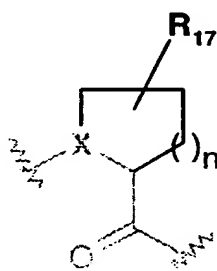
15   Formula III

R_2' being optionally substituted at any position with R_{17}, wherein **X** is CH or N; **n** is 1 or 2, and R_{17} is as defined below.

20

Most preferably, **X** is N. For example, preferably R_2' is propylene joined to **X** wherein **X** is nitrogen to form a proline substituted with R_{17} at P2.

Most preferably R_2' is the side chain of proline substituted at the 3-, 4-, or 5-position with R_{17}, wherein R_{17} is as defined below.
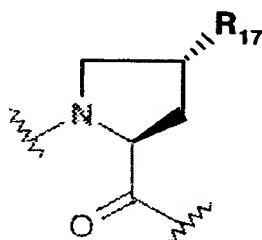
5   Still, most preferably R_2' is the side chain of proline (as shown in black) substituted with R_{17} at the 4-position with the stereochemistry shown in formula III':

Formula III'

10   wherein R_{17} is preferably OH; SH; NH_2; carboxyl; R_{12}; OR_{12}, SR_{12}, NHR_{12} or $NR_{12}R_{12}'$ wherein R_{12} and R_{12}' are independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl,

15   said alkyl or alkenyl optionally substituted with NH_2, OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom independently selected from the group consisting of: O, S, and N; or

20   R_{12} and R_{12}' are independently C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} alkyl, NH_2, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said aryl or aralkyl optionally containing at least one heteroatom

25   independently selected from the group consisting of: O, S, and N; said cyclic alkyl, cyclic alkenyl, aryl or aralkyl being optionally fused with a second 5-, 6- or 7-membered ring to form a cyclic system

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or heterocycle, said second ring being
optionally substituted with NH_2, OH, SH, halo,
carboxyl or carboxy(lower)alkyl; said second
ring optionally containing at least one
5   heteroatom independently selected from the group
consisting of: O, S, and N.

More preferably, R_{17} is OR_{12} wherein R_{12} is a C_6 or C_{10}
aryl or C_{7-16} aralkyl, said first aryl or aralkyl
10   optionally substituted with C_{1-6} alkyl, C_{3-7}
cycloalkyl, NH_2, OH, SH, halo, C_{1-6} alkoxy, carboxyl,
carboxy(lower)alkyl, or a second aryl or aralkyl;
said first and second aryl or aralkyl optionally
containing at least one heteroatom selected
15   independently from the group consisting of: O, S, and
N.

Most preferably, R_{17} is Bn; $PhCH_2CH_2$; $PhCH_2CH_2CH_2$; O-
Bn; o-tolylmethoxy; m-tolylmethoxy; p-tolylmethoxy;
20   1-naphtyloxy; 2-naphtyloxy; 1-naphthalenylmethoxy; 2-
naphthalenylmethoxy; (4-tert-butyl)methoxy; (3I-
Ph)CH_2O; (4Br-Ph)O; (2Br-Ph)O; (3Br-Ph)O; (4I-Ph)O;
(3Br-Ph)CH_2O; (3,5-Br_2-Ph)CH_2O;
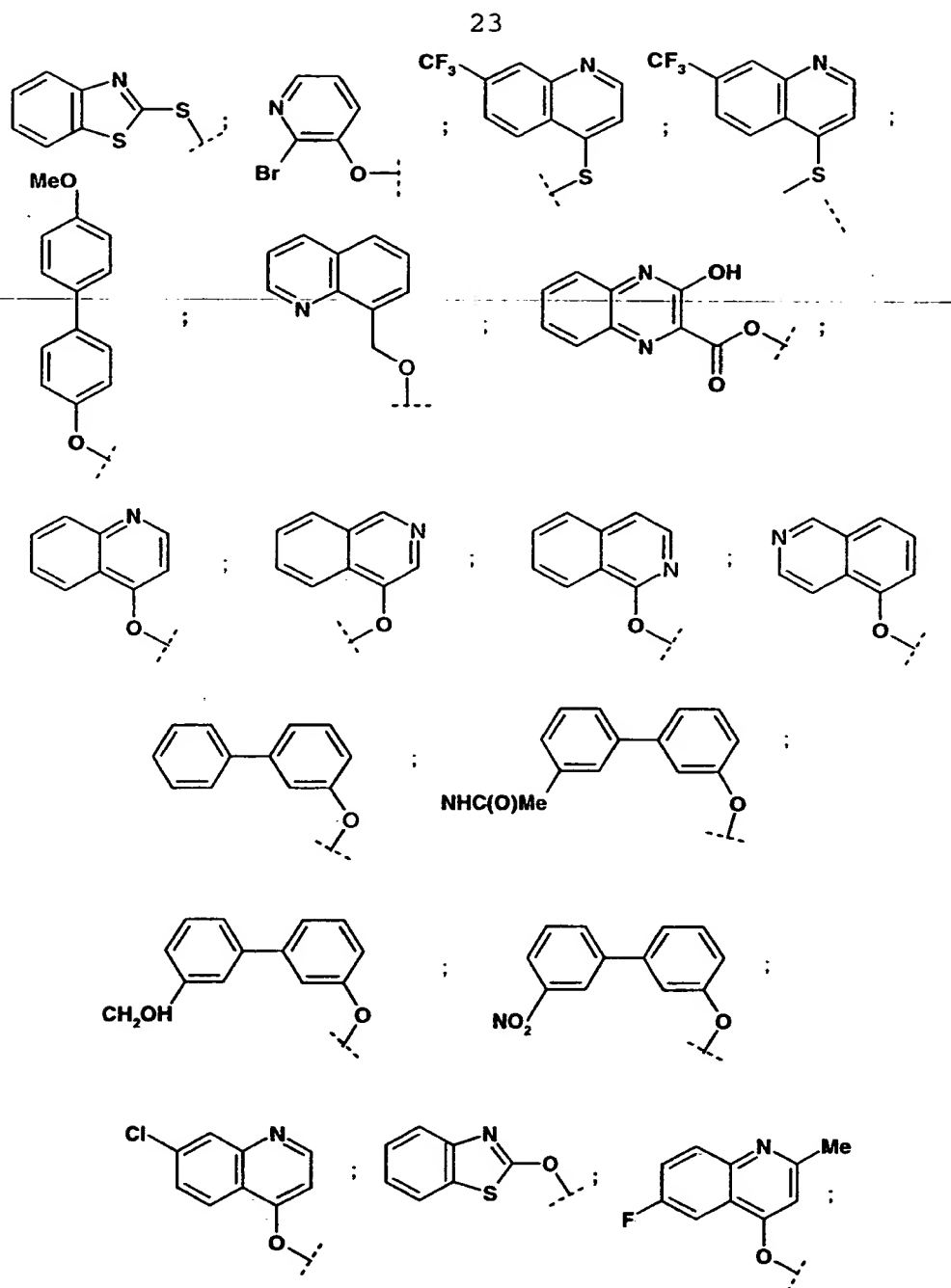
23

Still most preferably, **R₁₇** is Bn; PhCH₂CH₂;
PhCH₂CH₂CH₂; O-Bn; 1-naphtyloxy; 2-naphtyloxy; 1-
naphthalenylmethoxy; 2-naphthalenylmethoxy;

24
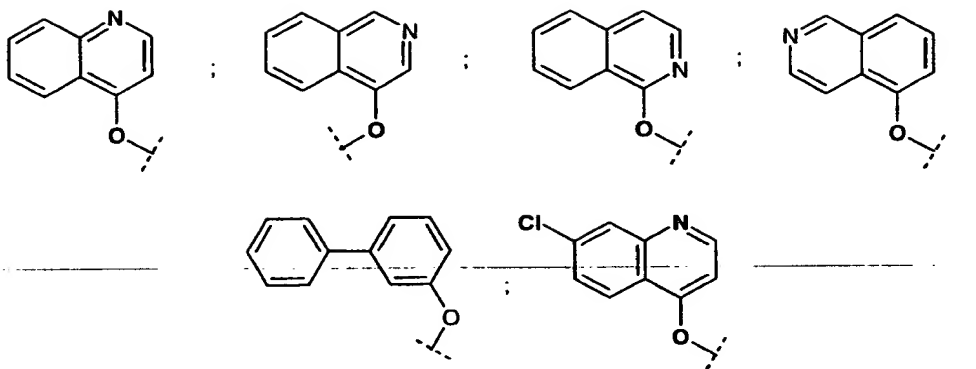
Even most preferably, R_{17} is O-Bn, 1-

naphthalenylmethoxy, or 2-naphthalenylmethoxy.

Preferably **Q** is:
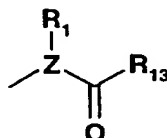
wherein **Z** is preferably CH or N.

Preferably, when **Z** is CH:

R_{13} is H; CF_3; CF_2CF_3; CH_2-R_{14}; $C(O)NH$-R_{14}, $NR_{14}R_{14'}$

wherein R_{14} and $R_{14'}$ are as defined above with the

proviso that R_{13} is not an α-amino acid or an ester

thereof. More preferably R_{13} is H; NH-R_{14} or C(O)NH-

R_{14}. Most preferably, R_{13} is H; or C(O)NH-R_{14}.

Preferably R_{14} is phenyl or C_{7-16} aralkyl. More

preferably, R_{14} is benzyl or CH(Me)Ph.

Alternatively, when **Z** is N:

R_{13} is preferably phenyl, or C_{7-16} aralkyl,

25
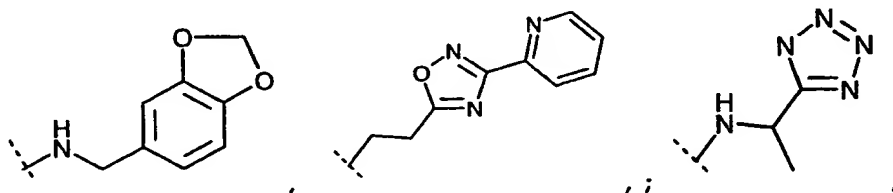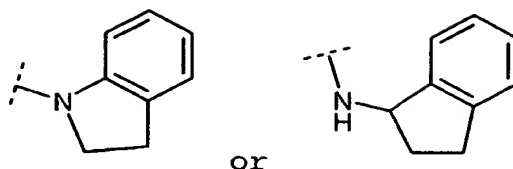
More preferably, R_{13} is naphthyl, $NH=CH(Me)Ph$, $NH=CH(Et)Ph$,

5
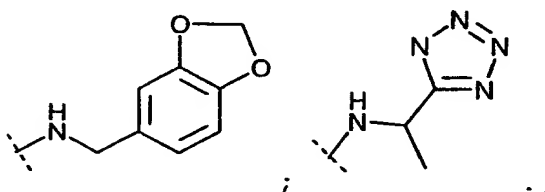
or   .
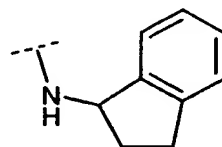
Most preferably, R_{13} is, $NH-CH(Me)Ph$, or
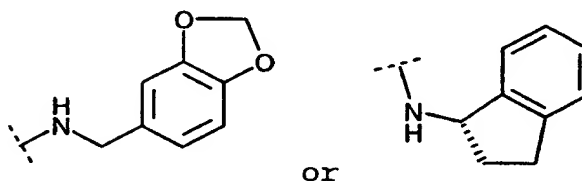
or   .

10

Alternatively, **Q** is preferably a phosphonate group of the formula:

wherein R_{15} and R_{16} are independently preferably C_{6-12}

15   aryloxy. More preferably, R_{15} and R_{16} are each phenoxy.

In all of the above cases, R_1 is preferably C_{1-6} alkyl or C_{1-6} alkenyl optionally substituted with halo.

More preferably, R_1 is C_{1-5} alkyl or C_{1-4} alkenyl optionally substituted with fluoro. Most preferably, R_1 is ethyl, propyl, isopentyl, or allyl.

5   According to an alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise an antiviral agent. Examples of antiviral agents include, ribavirin and amantadine.

10

According to another alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise other inhibitors of HCV protease.

15

According to yet another alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise an inhibitor of other targets in the HCV life cycle, such as helicase, polymerase,

20   or metalloprotease.

The pharmaceutical compositions of this invention may be administered orally, parenterally or via an implanted reservoir. We prefer oral administration

25   or administration by injection. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with

30   pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular,

intrasynovial, intrasternal, intrathecal, and
intralesional injection or infusion techniques.

5   The pharmaceutical compositions may be in the form of
a sterile injectable preparation, for example, as a
sterile injectable aqueous or oleaginous suspension.
This suspension may be formulated according to
techniques known in the art using suitable dispersing
or wetting agents (such as, for example. Tween 80)
10   and suspending agents.

The pharmaceutical compositions of this invention may
be orally administered in any orally acceptable
dosage form including, but not limited to, capsules,
15   tablets, and aqueous suspensions and solutions. In
the case of tablets for oral use, carriers which are
commonly used include lactose and corn starch.
Lubricating agents, such as magnesium stearate, are
also typically added. For oral administration in a
20   capsule form, useful diluents include lactose and
dried corn starch. When aqueous suspensions are
administered orally, the active ingredient is
combined with emulsifying and suspending agents. If
desired, certain sweetening and/or flavoring and/or
25   coloring agents may be added.

Other suitable vehicles or carriers for the above
noted formulations and compositions can be found in
standard pharmaceutical texts, e.g. in "Remington's
30   Pharmaceutical Sciences", The Science and Practice of
Pharmacy, 19th Ed. Mack Publishing Company, Easton,
Penn., (1995).

Dosage levels of between about 0.01 and about 100

28

mg/kg body weight per day, preferably between about
0.5 and about 75 mg/kg body weight per day of the
protease inhibitor compounds described herein are
useful in a monotherapy for the prevention and
5   treatment of HCV mediated disease. Typically, the
pharmaceutical compositions of this invention will be
administered from about 1 to about 5 times per day or
alternatively, as a continuous infusion. Such
administration can be used as a chronic or acute
10   therapy. The amount of active ingredient that may be
combined with the carrier materials to produce a
single dosage form will vary depending upon the host
treated and the particular mode of administration. A
typical preparation will contain from about 5% to
15   about 95% active compound (w/w). Preferably, such
preparations contain from about 20% to about 80%
active compound.

As the skilled artisan will appreciate, lower or
20   higher doses than those recited above may be
required. Specific dosage and treatment regimens for
any particular patient will depend upon a variety of
factors, including the activity of the specific
compound employed, the age, body weight, general
25   health status, sex, diet, time of administration,
rate of excretion, drug combination, the severity and
course of the infection, the patient's disposition to
the infection and the judgment of the treating
physician. Generally, treatment is initiated with
30   small dosages substantially less than the optimum
dose of the peptide. Thereafter, the dosage is
increased by small increments until the optimum
effect under the circumstances is reached. In
general, the compound is most desirably administered

at a concentration level that will generally afford antivirally effective results without causing any harmful or deleterious side effects.

5   When the compositions of this invention comprise a combination of a compound of formula I and one or more additional therapeutic or prophylactic agent, both the compound and the additional agent should be present at dosage levels of between about 10 to 100%,
10   and more preferably between about 10 and 80% of the dosage normally administered in a monotherapy regimen.

     When these compounds or their pharmaceutically
15   acceptable salts are formulated together with a pharmaceutically acceptable carrier, the resulting composition may be administered *in vivo* to mammals, such as man, to inhibit HCV NS3 protease or to treat or prevent HCV virus infection. Such treatment may
20   also be achieved using the compounds of this invention in combination with agents which include, but are not limited to: immunomodulatory agents, such as α-, β-, or γ-interferons; other antiviral agents such as ribavirin, amantadine; other
25   inhibitors of HCV NS3 protease; inhibitors of other targets in the HCV life cycle such as helicase, polymerase, metalloprotease, or internal ribosome entry; or combinations thereof. The additional agents may be combined with the compounds of this
30   invention to create a single dosage form. Alternatively these additional agents may be separately administered to a mammal as part of a multiple dosage form.

30

Accordingly, another embodiment of this invention provides methods of inhibiting HVC NS3 protease activity in mammals by administering a compound of the formula I, wherein the substituents are as

5   defined above.

In a preferred embodiment, these methods are useful in decreasing HCV NS3 protease activity in a mammal. If the pharmaceutical composition comprises only a

10 compound of this invention as the active component, such methods may additionally comprise the step of administering to said mammal an agent selected from an immunomodulatory agent, an antiviral agent, a HCV protease inhibitor, or an inhibitor of other targets

15 in the HCV life cycle such as helicase, polymerase, or metallo protease. Such additional agent may be administered to the mammal prior to, concurrently with, or following the administration of the compositions of this invention.

20

In an alternate preferred embodiment, these methods are useful for inhibiting viral replication in a mammal. Such methods are useful in treating or preventing HCV disease. If the pharmaceutical

25 composition comprises only a compound of this invention as the active component, such methods may additionally comprise the step of administering to said mammal an agent selected from an immunomodulatory agent, an antiviral agent, a HCV

30 protease inhibitor, or an inhibitor of other targets in the HCV life cycle. Such additional agent may be administered to the mammal prior to, concurrently with, or following the administration of the composition according to this invention.

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The compounds set forth herein may also be used as
laboratory reagents. The compounds of this invention
may also be used to treat or prevent viral
5   contamination of materials and therefore reduce the
risk of viral infection of laboratory or medical
personnel or patients who come in contact with such
materials (e.g. blood, tissue, surgical instruments
and garments, laboratory instruments and garments,
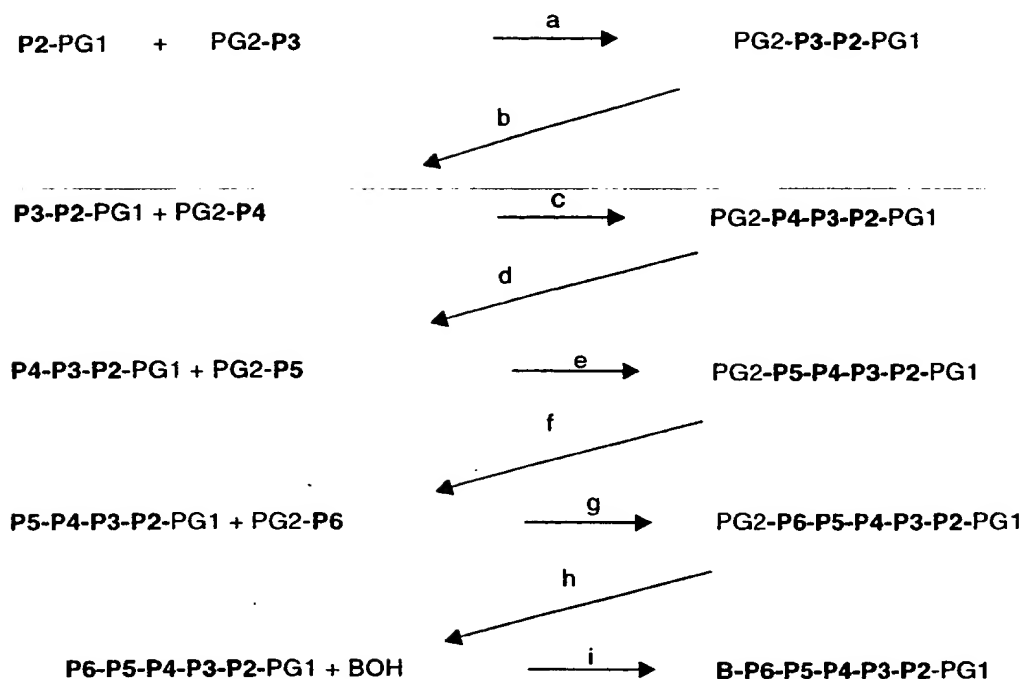10 and blood collection apparatuses and materials).

PROCESS

Synthesis of P6-P2 fragments

The P2-P6 fragments of the compounds of the present
15 invention were synthesized according to the process
as illustrated in scheme I (wherein PG1 is a carboxyl
protecting group and PG2 is an amino protecting
group):

32

Scheme I

P2-PG1   +   PG2-P3 $\xrightarrow{\text{a}}$ PG2-**P3**-**P2**-PG1

$\xleftarrow{\text{b}}$

P3-P2-PG1 + PG2-**P4** $\xrightarrow{\text{c}}$ PG2-**P4-P3-P2**-PG1

$\xleftarrow{\text{d}}$

P4-P3-P2-PG1 + PG2-**P5** $\xrightarrow{\text{e}}$ PG2-**P5-P4-P3-P2**-PG1

$\xleftarrow{\text{f}}$

P5-P4-P3-P2-PG1 + PG2-**P6** $\xrightarrow{\text{g}}$ PG2-**P6-P5-P4-P3-P2**-PG1

$\xleftarrow{\text{h}}$

P6-P5-P4-P3-P2-PG1 + BOH $\xrightarrow{\text{i}}$ **B-P6-P5-P4-P3-P2**-PG1

Briefly, the P2, P3, P4, and optionally P5 and P6 can
be linked by well known peptide coupling techniques.

5   The P2, P3, P4, and P5 and P6 moieties may be linked
together in any order as long as the final compound
corresponds to peptides of formula I. For example, P6
can be linked to P5 to give P5-P6 that is linked to
P4-P3-P2; or P6 linked to P5-P4-P3 then linked to an

10   appropriately C-terminal protected P2.

Generally, peptides are elongated by deprotecting the
α-amino group of the N-terminal residue and coupling
the unprotected carboxyl group of the next suitably

15   N-protected amino acid through a peptide linkage
using the methods described. This deprotection and
coupling procedure is repeated until the desired
sequence is obtained. This coupling can be performed
with the constituent amino acids in stepwise fashion,

as depicted in Scheme I, or by condensation of
fragments (two or several amino acids), or
combination of both processes, or by solid phase
peptide synthesis according to the method originally
5   described in Merrifield, J. Am. Chem. Soc., (1963),
     85, 2149-2154, the disclosure of which is hereby
     incorporated by reference.

     Coupling between two amino acids, an amino acid and a
10   peptide, or two peptide fragments can be carried out
     using standard coupling procedures such as the azide
     method, mixed carbonic-carboxylic acid anhydride
     (isobutyl chloroformate) method, carbodiimide
     (dicyclohexylcarbodiimide, diisopropylcarbodiimide,
15   or water-soluble carbodiimide) method, active ester
     (p-nitrophenyl ester, N-hydroxysuccinic imido ester)
     method, Woodward reagent K-method,
     carbonyldiimidazole method, phosphorus reagents or
     oxidation-reduction methods. Some of these methods
20   (especially the carbodiimide method) can be enhanced
     by adding 1-hydroxybenzotriazole. These coupling
     reactions can be performed in either solution (liquid
     phase) or solid phase.

25   More explicitly, the coupling step involves the
     dehydrative coupling of a free carboxyl of one
     reactant with the free amino group of the other
     reactant in the presence of a coupling agent to form
     a linking amide bond. Description of such coupling
30   agents are found in general textbooks on peptide
     chemistry, for example, M. Bodanszky, "Peptide
     Chemistry", 2nd rev ed., Springer-Verlag, Berlin,
     Germany, (1993). Examples of suitable coupling
     agents are *N,N'*-dicyclohexylcarbodiimide, 1-

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hydroxybenzotriazole in the presence of N,N'-
dicyclohexylcarbodiimide or N-ethyl-N'-[(3-
dimethylamino)propyl]carbodiimide.   A very practical
and useful coupling agent is the commercially

5   available (benzotriazol-1-yloxy)tris-
(dimethylamino)phosphonium hexafluorophosphate,
either by itself or in the presence of 1-
hydroxybenzotriazole.   Another very practical and
useful coupling agent is commercially available 2-

10   (1H-benzotriazol-1-yl)-N, N, N', N'-
tetramethyluronium tetrafluoroborate. Still another
very practical and useful coupling agent is
commercially available O-(7-azabenzotriazol-1-yl)-
N,N,N',N'-tetramethyluronium hexafluorophosphate.

15

The coupling reaction is conducted in an inert
solvent, e.g. dichloromethane, acetonitrile or
dimethylformamide.   An excess of a tertiary amine,
e.g. diisopropylethylamine, N-methylmorpholine or N-

20   methylpyrrolidine, is added to maintain the reaction
mixture at a pH of about 8.   The reaction temperature
usually ranges between 0°C and 50°C and the reaction
time usually ranges between 15 min and 24 h.

25   When a solid phase synthetic approach is employed,
the C-terminal carboxylic acid is attached to an
insoluble carrier (usually polystyrene).   These
insoluble carriers contain a group that will react
with the carboxylic group to form a bond that is

30   stable to the elongation conditions but readily
cleaved later.   Examples of which are: chloro- or
bromomethyl resin, hydroxymethyl resin, and
aminomethyl resin. Many of these resins are
commercially available with the desired C-terminal

amino acid already incorporated. In addition to the foregoing, other methods of peptide synthesis are described in Stewart and Young, "Solid Phase Peptide Synthesis", 2nd ed., Pierce Chemical Co., Rockford,

5   IL (1984); Gross, Meienhofer, Udenfriend, Eds., "The Peptides: Analysis, Synthesis, Biology", Vol. 1, 2, 3, 5, and 9, Academic Press, New-York, (1980-1987); Bodansky et al., "The Practice of Peptide Synthesis" Springer-Verlag, New-York (1984), the disclosures of

10   which are hereby incorporated by reference.

The functional groups of the constituent amino acids generally must be protected during the coupling reactions to avoid formation of undesired bonds. The

15   protecting groups that can be used are listed in Greene, "Protective Groups in Organic Chemistry", John Wiley & Sons, New York (1981) and "The Peptides: Analysis, Synthesis, Biology", Vol. 3, Academic Press, New York (1981), the disclosures of which are

20   hereby incorporated by reference.

The α-carboxyl group of the C-terminal residue is usually protected as an ester (PG1) that can be cleaved to give the carboxylic acid. Protecting

25   groups that can be used include: 1) alkyl esters such as methyl, trimethylsilylethyl and t-butyl, 2) aralkyl esters such as benzyl and substituted benzyl, or 3) esters that can be cleaved by mild base treatment or mild reductive means such as

30   trichloroethyl and phenacyl esters.

The α-amino group of each amino acid to be coupled to the growing peptide chain must be protected (PG2). Any protecting group known in the art can be used.

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Examples of such groups include: 1) acyl groups such as formyl, trifluoroacetyl, phthalyl, and *p*-toluenesulfonyl; 2) aromatic carbamate groups such as benzyloxycarbonyl (Cbz or Z) and substituted benzyloxycarbonyls, and 9-fluorenylmethyloxycarbonyl (Fmoc); 3) aliphatic carbamate groups such as *tert*-butyloxycarbonyl (Boc), ethoxycarbonyl, diisopropylmethoxycarbonyl, and allyloxycarbonyl; 4) cyclic alkyl carbamate groups such as cyclopentyloxycarbonyl and adamantyloxycarbonyl; 5) alkyl groups such as triphenylmethyl and benzyl; 6) trialkylsilyl such as trimethylsilyl; and 7) thiol containing groups such as phenylthiocarbonyl and dithiasuccinoyl. The preferred α-amino protecting group is either Boc or Fmoc. Many amino acid derivatives suitably protected for peptide synthesis are commercially available.

The α-amino protecting group of the newly added amino acid residue is cleaved prior to the coupling of the next amino acid. When the Boc group is used, the methods of choice are trifluoroacetic acid, neat or in dichloromethane, or HCl in dioxane or in ethyl acetate. The resulting ammonium salt is then neutralized either prior to the coupling or *in situ* with basic solutions such as aqueous buffers, or tertiary amines in dichloromethane or acetonitrile or dimethylformamide. When the Fmoc group is used, the reagents of choice are piperidine or substituted piperidine in dimethylformamide, but any secondary amine can be used. The deprotection is carried out at a temperature between 0°C and room temperature (RT).

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Any of the amino acids having side chain
functionalities must be protected during the
preparation of the peptide using any of the above-
described groups. Those skilled in the art will
5   appreciate that the selection and use of appropriate
protecting groups for these side chain
functionalities depend upon the amino acid and
presence of other protecting groups in the peptide.
The selection of such protecting groups is important
10   in that the group must not be removed during the
deprotection and coupling of the α-amino group.

For example, when Boc is used as the α-amino
protecting group, *p*-toluenesulfonyl (tosyl) is
15   suitable to protect the amino side chain of amino
acids such as Lys and Arg; acetamidomethyl, benzyl
(Bn), or *t*-butylsulfonyl moieties can be used to
protect the sulfide containing side chain of
cysteine; benzyl (Bn) ethers can be used to protect
20   the hydroxy containing side chains of serine,
threonine or hydroxyproline; and benzyl esters can be
used to protect the carboxy containing side chains of
aspartic acid and glutamic acid.

25   When Fmoc is chosen for the α-amine protection,
usually *tert*-butyl based protecting groups are
acceptable. For instance, Boc can be used for lysine
and arginine, *tert*-butyl ether for serine, threonine
and hydroxyproline, and *tert*-butyl ester for aspartic
30   acid and glutamic acid. Triphenylmethyl (Trityl)
moiety can be used to protect the sulfide containing
side chain of cysteine.

Once the elongation of the peptide is completed all of the protecting groups are removed. When a liquid phase synthesis is used, the protecting groups are removed in whatever manner is dictated by the choice

5 of protecting groups. These procedures are well known to those skilled in the art.

When a solid phase synthesis is used, the peptide is cleaved from the resin simultaneously with the

10 removal of the protecting groups. When the Boc protection method is used in the synthesis, treatment with anhydrous HF containing additives such as dimethyl sulfide, anisole, thioanisole, or p-cresol at 0°C is the preferred method for cleaving the

15 peptide from the resin. The cleavage of the peptide can also be accomplished by other acid reagents such as trifluoromethanesulfonic acid/ trifluoroacetic acid mixtures. If the Fmoc protection method is used the N-terminal Fmoc group is cleaved with reagents

20 described earlier. The other protecting groups and the peptide are cleaved from the resin using solution of trifluoroacetic acid and various additives such as anisole, etc.

25 **Synthesis of capping group B and P6, P5, P4, and P3 moieties**

Different capping groups **B** are introduced to protected P6, P5, P4, the whole peptide or to any peptide segment with an appropriate acyl chloride

30 that is either available commercially or for which the synthesis is well known in the art.
Different **P6** to **P3** moieties are available commercially or the synthesis is well known in the art.
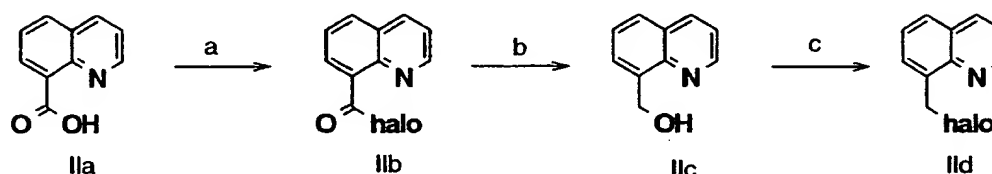
Synthesis of P2 moieties

1. Synthesis of precursors:

5   A) Synthesis of haloarylmethane derivatives.
         The preparation of halomethyl-8-quinoline **IId**
         was done according to the procedure of K.N.
         Campbell et al., J. Amer. Chem. Soc., (1946),
   68, 1844.

10   Scheme II

         Briefly, 8-quinoline carboxylic acid **IIa** was
         converted to the corresponding alcohol **IIc** by
         reduction of the corresponding acyl halide **IIb**
15   with a reducing agent such as lithium aluminium
         hydride. Treatment of alcohol **IIb** with the
         appropriate hydrohaloacid gives the desired halo
         derivative **IId.** A specific embodiment of this
         process is presented in Example 1.

20

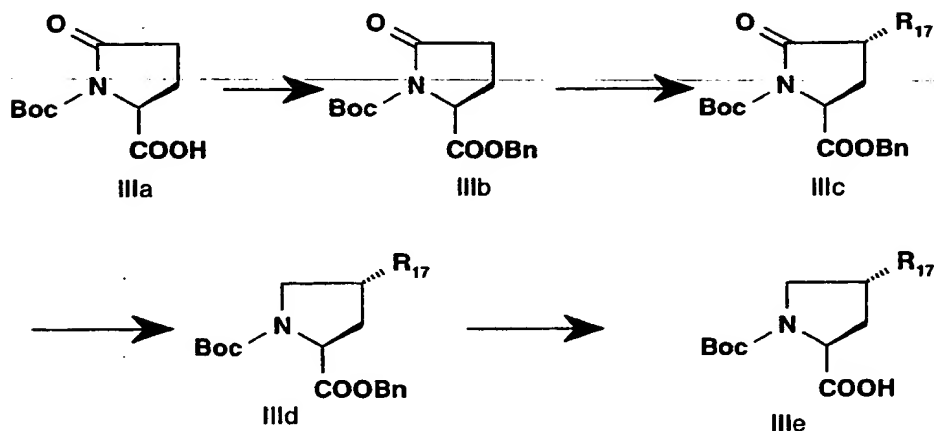## 2. Synthesis of P2:

A) The synthesis of 4-substituted proline (wherein
R_{17} is attached to the ring via a carbon atom) (with
25   the stereochemistry as shown):
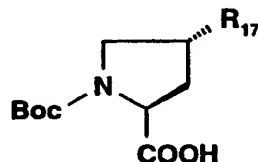
         is done as shown in Scheme III according to the
   procedures described by J. Ezquerra et al.

40

(Tetrahedron, (1993), <u>38</u>, 8665-8678) and C.
Pedregal et al. (Tetrahedron Lett., (1994), <u>35</u>,
2053-2056).

Scheme III

IIIa   IIIb   IIIc

IIId   IIIe

5

Briefly, Boc-pyroglutamic acid is protected as a
benzyl ester. Treatment with a strong base such
as lithium diisopropylamide followed by addition
of an alkylating agent (Br-R_{17} or I-R_{17}) gives

10   the desired compounds **IIIe** after reduction of
the amide and deprotection of the ester. A
specific embodiment of this process is presented
in Example 2.

15   B) The synthesis of O-alkylated 4-*(R)*-hydroxyproline:
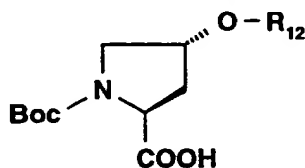

may be carried out using the different processes
described below.

20   B.1) When R_{12} is aralkyl, the process can be
carried out according to the procedure described
by E.M. Smith et al. (J. Med. Chem. (1988), <u>31</u>,
875-885). Briefly, commercially available Boc-

41

4 *(R)*-hydroxyproline is treated with a base such as sodium hydride and the resulting alkoxide reacted with an alkylating agent (Br-R_{12} or I-R_{12}) to give the desired compounds. Specific

5   embodiments of this process are presented in Examples 3 and 4.

B.2) When R_{12} is aryl, the compounds can be prepared via a Mitsunobu reaction (Mitsunobu

10   (1981), Synthesis, <u>January</u>, 1-28; Rano *et al.*, (1995), Tet. Lett. <u>36(22)</u>, 3779-3792; Krchnak *et al.*, (1995), Tet. Lett. <u>36(5)</u>, 62193-6196; Richter *et al.*, (1994), Tet. Lett. <u>35(27)</u>, 4705-4706). Briefly, commercially available Boc-

15   4 *(S)*-hydroxyproline methyl ester is treated with the appropriate aryl alcohol or thiol in the presence of triphenylphosphine and diethylazodicarboxylate (DEAD) and the resulting ester is hydrolysed to the acid. Specific

20   embodiment of this process are presented in Example 5.

Scheme IV

IVa   IVb
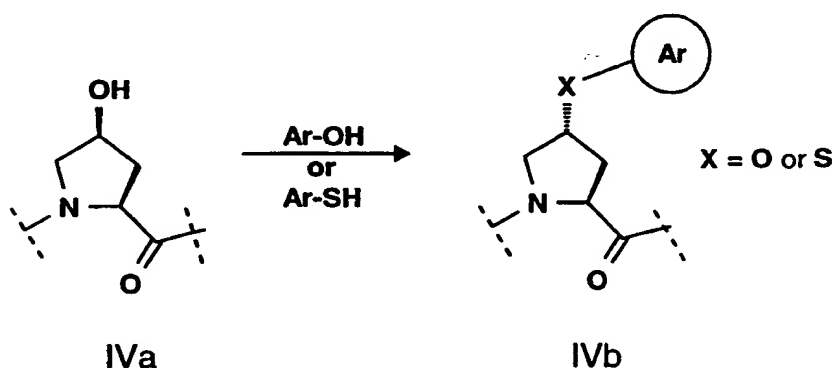
25   Alternatively,   the   Mitsunobu   reaction   can   be performed   on   solid   phase   (as   shown   in   Scheme   IV). The   96-well   block   of   the   Model   396   synthesizer

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(advanced ChemTech) is provided with aliquots of resin-bound compound (**IVa**) and a variety of aryl alcohols or thiols and appropriate reagents are added. After incubation, each resin-bound product

5   (**IVb**) is washed, dried, and cleaved from the resin.

B.2.a)   A Suzuki reaction (Miyaura *et al.*,
(1981), Synth. Comm. <u>11</u>, 513; Sato *et al.*,
(1989), Chem. Lett., 1405; Watanabe *et al.*,

10   (1992), Synlett., 207; Takayuki *et al.*,
(1993), J. Org. Chem. <u>58</u>, 2201; Frenette *et al.*, (1994), Tet. Lett. <u>35(49)</u>, 9177-9180;
Guiles *et al.*, (1996), J. Org. Chem. <u>61</u>,
5169-5171) can also be used to further

15   functionalize the aryl substituent.

**C) Synthesis of compounds of formula I wherein Q is:**
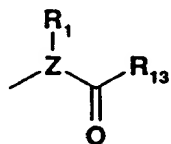
20   wherein **Z** is CH; **R₁** is as defined above and **R₁₃** is
CF_3, CF_2CF_3 or $C(O)NH\text{-}\mathbf{R_{14}}$; was done as described in
Scheme VIII.

The synthesis of the required P1 moieties was done as

25   follows:

i) For the synthesis of trifluoromethyl alcohols of
formula **Vd** the procedure described by J.W. Skiles
et al. (J. Med. Chem. (1992), <u>35</u>, 641-662) was

30   used as illustrated:

43

Scheme V
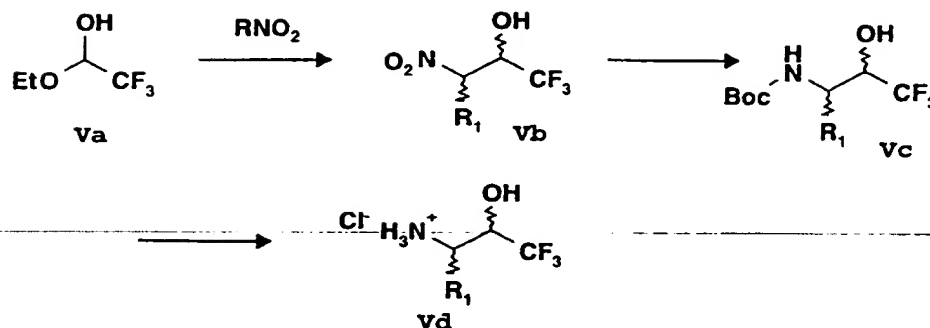
wherein R_1 is as defined above.

5   Briefly, a condensation between commercially
        available trifluoroacetaldehyde ethyl hemiacetal **Va**
        and the appropriate nitroalkane affords the
        corresponding nitroalcohol **Vb**. The nitro group was
        reduced (preferably with Ra-Ni) and protected as the
10   Boc-derivative **Vc** to allow easier purification of the
        fragment. Treatment of the Boc-amine with anhydrous
        HCl affords the hydrochloride salt **Vd**.

        ii) For the synthesis of pentafluoroethyl alcohols of
15   formula **VId**, the procedure described by M.R.
        Angelastro et al. (J. Med. Chem., (1994), <u>37</u>, 4538-
   4554) was used as illustrated in scheme VI:

Scheme VI
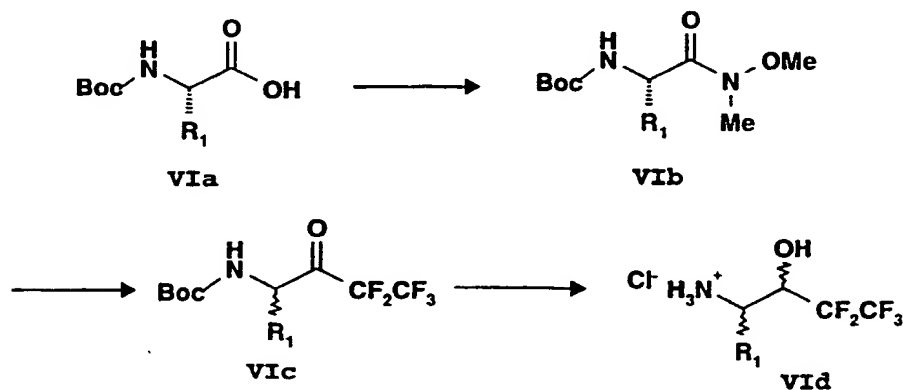
20   wherein R_1 is as defined above.

44

Briefly, the Boc-amino acid **VIa** was converted to the
Weinreb amide **VIb** according to the procedure
described by Castro, B. et al. (Synthesis, (1983),
676-678) and treated with lithium pentafluoroethane.

5   The resulting pentafluoroethyl ketone **VIc** was reduced
and the Boc protecting group removed with anhydrous
HCl to give the hydrochloride salt of the desired
amino alcohol **VId**.

10   iii) For the synthesis of hydroxy amides of formula
VIIf the procedure described by Peet *et al*. (Tet.
Lett. (1988), 3433) was used as illustrated in scheme
VII:

Scheme VII

15
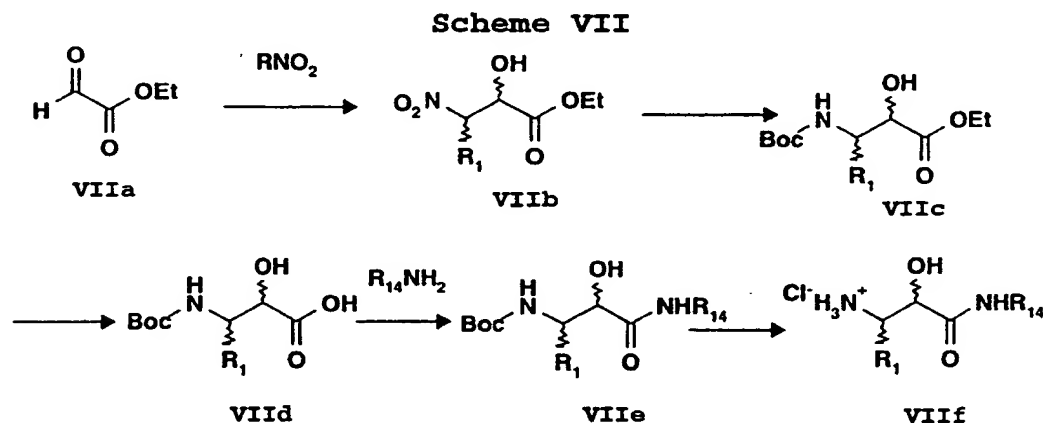
wherein **R₁** is as defined above.

Briefly, condensation between ethyl glyoxylate **VIIa**
20   and a nitroalkane under basic conditions afforded the
corresponding nitroalcohol **VIIb**. The nitro group was
reduced (preferably with Ra-Ni) and protected as the
Boc-derivative **VIIc**. Saponification of the ester
group followed by standard coupling with an amine
25   gave the hydroxy amide **VIIe**. Removal of the Boc
protecting group with anhydrous HCl afforded the

hydrochloride salt of the desired amino hydroxyamide
VIIf.

iv) The coupling to P2-P6 was carried out as
illustrated in scheme VIII:

Scheme VIII
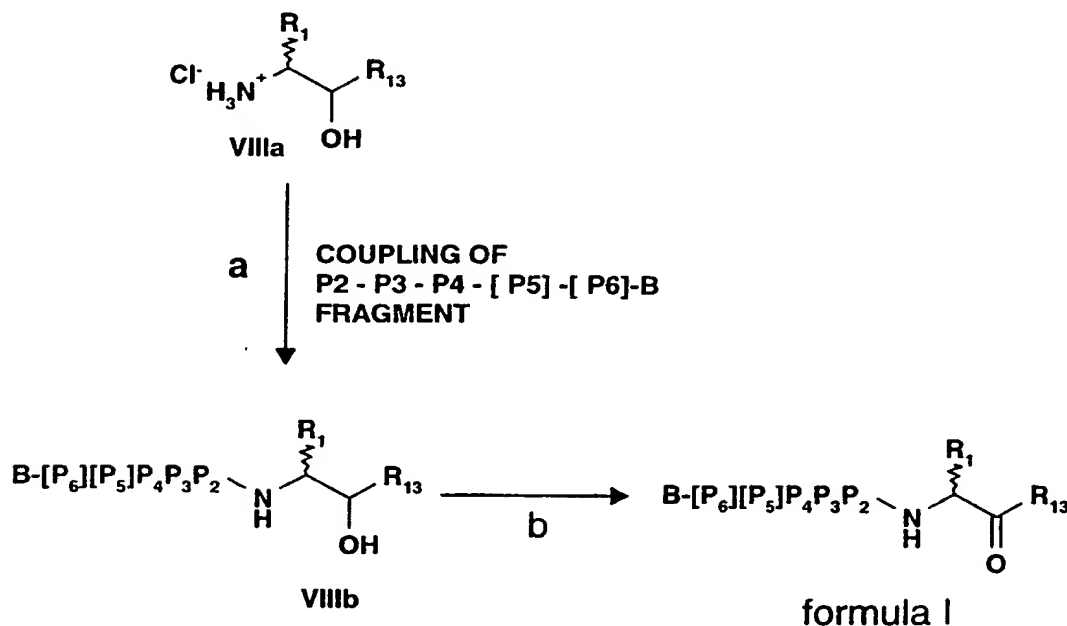
10   a) The P6 to P2 fragment can be linked to the free
amino group of the amino alcohol derivative **VIIIa** as
described previously in Scheme I to give the peptido
alcohol **VIIIb.**

15   b) The alcohol functionality of the peptido alcohol
VIIIb is then oxidized by techniques and procedures
well known and appreciated by one of ordinary skill
in the art, such as the Swern Oxidation (Tidwell,
T.T., Synthesis, (1990), 857-870), or more
20   specifically the Pfitzner-Moffatt oxidation (K. E.
Pfitzner, and J. G. Moffatt, J. Am. Chem. Soc.,
(1965), 5670-5678) and the Dess-Martin periodinane
method (D. B. Dess or J.C. Martin, (J. Org. Chem.,

(1983), 48, 4155-4156) to give the compounds of
formula I wherein Q contains an activated carbonyl.

**D) Synthesis of compounds of formula I wherein Q is:**

$$\begin{array}{c} R_1 \\ | \\ Z \\ \diagdown \diagup R_{13} \\ \| \\ O \end{array}$$
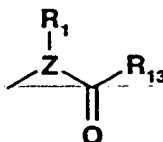
5

wherein Z is N; and R_{13} is NHR_{14}, $NR_{14}R_{14}'$, CH_2-R_{14},
$CHR_{14}R_{14}'$ or $O-R_{14}$; wherein R_{14} and R_1 are as defined
above, was done as described in scheme X.

10   i) For the synthesis of the aza-containing P1
      fragments, the procedure described by A. S. Dutta
      et al. (J. Chem. Soc. Perkin I, (1975), 1712) was
      followed as illustrated:

15   **Scheme IX**
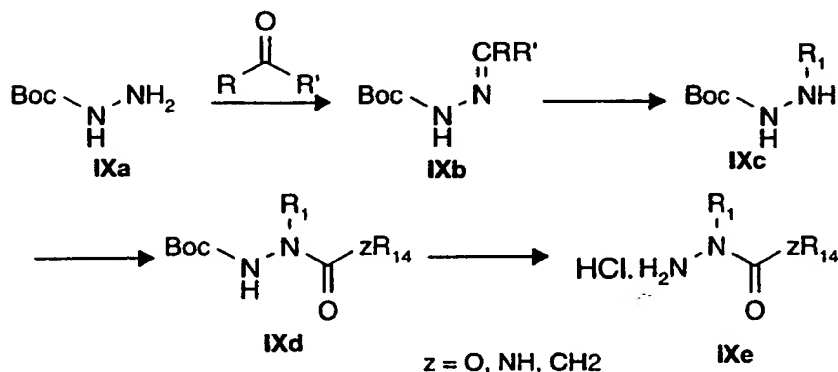
wherein R_1 and R_{14} are as defined hereinabove.

Briefly, commercially available Boc hydrazine **IXa** was
20   treated with an appropriate aldehyde or ketone to
      afford the corresponding hydrazone **IXb**. The hydrazone
      was reduced (preferentially with DIBAL) to give the
      alkyl carbazate **IXc**. Treatment of the alkyl carbazate
      with isocyanates affords the corresponding aza
25   peptide fragment (**IXd**, wherein z=NH). Treatment of

the alkyl carbozate with carbamoyl chlorides afford the corresponding aza peptide fragment (**IXd**, wherein $z=NR_{14}R_{14'}$). Treatment of the alkyl carbazate with chloroformates afford the aza-carbamate (**IXd**, wherein

5   $z=O$) while treatment with acid chlorides affords the carbon analogues (**IXd**, wherein $z=CH_2$). Alternatively, treatment of the alkyl carbazate with carboxylic acids using standard coupling conditions affords the corresponding aza peptide fragment (**IXd**,

10   wherein $Z = CHR_{14'}$ or CH_2). Finally the Boc-protecting group was removed with anhydrous HCl to give the desired aza-derivatives **IXe.**
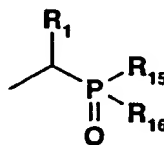

ii) Coupling of P2-P6 was carried out according to

15   scheme X:
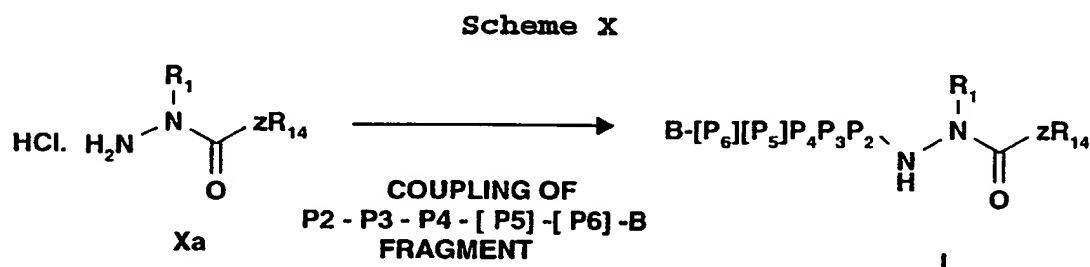
Scheme X

wherein z is O, NH, CH_2, $CHR_{14'}$ or $NR_{14'}$.

20

The P6 to P2 fragment can be linked to the free amino group of derivative **Xa** as described previously in Scheme I to give the aza-derivatives of formula I wherein **Z** is N.
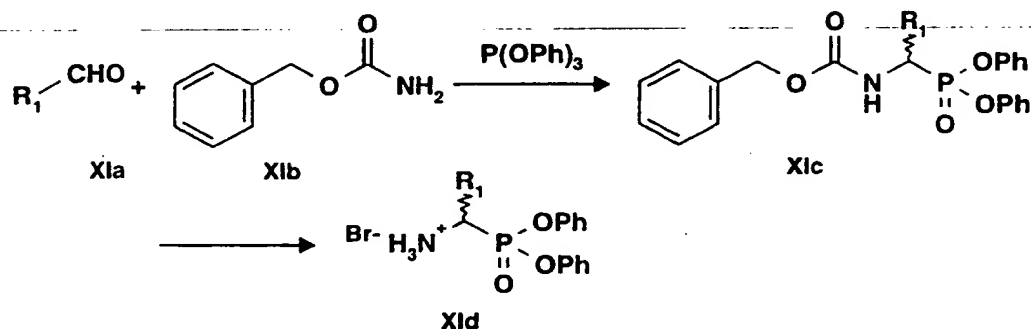
25

E) Synthesis of compounds of formula I wherein Q is:

48

wherein R_{15} and R_{16} are as defined above, the procedure described by J. Oleksyszyn et al. (Synthesis, (1979), 985-986) was used as illustrated in scheme XI:

5

Scheme XI

wherein R_1 is as defined hereinabove.

Briefly, a two step synthesis of the diphenyl ester

10   was accomplished by condensation of a suitable aldehyde **XIa,** benzyl carbamate **XIb,** and triphenyl phosphite in the presence of acetic acid. Removal of the benzyloxycarbonyl protecting group of **XIc** using HBr/AcOH afforded the desired hydrobromide salt **XId.**

15

The P6 to P2 fragment can be linked to the free amino group of phosphonate derivative of formula **XId** as described previously in Scheme I to give the phophono-peptide of formula I wherein **Q** is a

20   phosphonate moiety.

The following examples are provided to describe the invention in further detail. These examples, which set forth the best mode presently contemplated for

25   carrying out the invention, are intended to illustrate and not to limit the invention.

Examples

49

The present invention is illustrated in further detail by the following non-limiting examples.

Temperatures are given in degrees Celsius (°C).
5   Solution percentages express a weight to volume relationship, and solution ratios express a volume to volume relationship, unless stated otherwise. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer; the
10   chemical shifts (δ) are reported in parts per million. Flash chromatography was carried out on silica gel (SiO_2) according to Still's flash chromatography technique (W.C. Still et al., J. Org. Chem., (1978), 43, 2923).
15

Abbreviations used in the examples include Bn: benzyl; Boc: *tert*-butyloxycarbonyl {$Me_3COC(O)$}; BSA: bovine serum albumin; CHAPS: 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; DBU: 1,8-
20   diazabicyclo[5.4.0]undec-7-ene; CH_2Cl_2= DCM: methylene chloride; DIAD: Diisopropyl azodicarboxylate; DIPEA: diisopropylethylamine; DMAP: dimethylaminopyridine; DCC: 1,3-dicyclohexyl-carbodiimide; DME: 1,2-dimethyoxyethane; DMF:
25   dimethylformamide; DMSO: dimethylsulfoxide; DTT: dithiothreitol or threo-1,4-dimercapto-2,3-butanediol; EDTA: ethylenediaminetetraacetic acid; Et: ethyl; EtOH: ethanol; EtOAc: ethyl acetate; Et_2O: diethyl ether; HPLC: high performance liquid
30   chromatography; MS: mass spectrometry (MALDI-TOF: Matrix Assisted Laser Disorption Ionisation-Time of Flight, FAB: Fast Atom Bombardment);LAH: lithium aluminum hydride; Me: methyl; MeOH: methanol; MES: (2-{*N*-morpholino}ethane-sulfonic acid); NaHMDS:

50

sodium bis(trimethylsilyl)amide; NMM: *N*-
methylmorpholine; NMP: *N*-methylpyrrolidine; Pr:
propyl; Succ: 4-hydroxy-1,4-dioxobutyl; PNA: 4-
nitrophenylamino or p-nitroanalide; TBAF: tetra-*n*-

5   butylammonium fluoride; TCEP: tris(2-carboxyethyl)
phosphine hydrochloride; TFA: trifluoroacetic acid;
THF: tetrahydrofuran; TIS: triisopropylsilane; TLC:
thin layer chromatography; TMSE: trimethylsilylethyl;
Tris/HCl: tris(hydroxymethyl)aminomethane

10   hydrochloride.

Example 1

Synthesis of bromomethyl-8-quinoline (1):

(1)
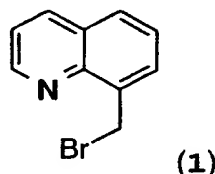
15   To commercially available 8-quinoline carboxylic acid
(2.5 g, 14.4 mmol) was added neat thionyl chloride
(10 ml, 144 mmol). This mixture was heated at 80°C
for 1 h before the excess thionyl chloride was
distilled off under reduced pressure. To the

20   resulting brownish solid was added absolute EtOH (15
mL) which was heated at 80°C for 1 h before being
concentrated *in vacuo*. The residue was partitioned
between EtOAc and saturated aqueous NaHCO$_3$, and the
organic phase dried (MgSO$_4$), filtered and

25   concentrated to give a brownish oil (2.8 g). This
material (ca. 14.4 mmol) was added dropwise over 35
min to a LAH (0.76 g, 20.2 mmol)/Et$_2$O suspension
which was cooled to -60°C. The reaction mixture was
slowly warmed to -35°C over 1.5 h before the reaction

30   was complete. The reaction was quenched with
MgSO$_4$.10H$_2$O slowly over 30 min and then wet THF. The

mixture was partitioned between Et$_2$O and 10% aqueous
NaHCO$_3$. The organic phase was dried (MgSO$_4$), filtered
and concentrated to give a yellowish solid (2.31 g,
80% over 2 steps) corresponding to the alcohol. The
5   alcohol (2.3 g, 11.44 mmol) was dissolved in AcOH/HBr
(20 mL, 30% solution from Aldrich) and heated at 70°C
for 2.5 h. The mixture was concentrated *in vacuo* to
dryness, partitioned between EtOAc (100 mL) and
saturated aqueous NaHCO$_3$ before being dried (MgSO$_4$),
10   filtered and concentrated to give the desired
compound (**1**) as a brownish solid (2.54 g, 100%).

Example 2

Synthesis of Boc-4(R)-(3-phenylpropyl)proline (2d).

15

2a   2b

2c   2d
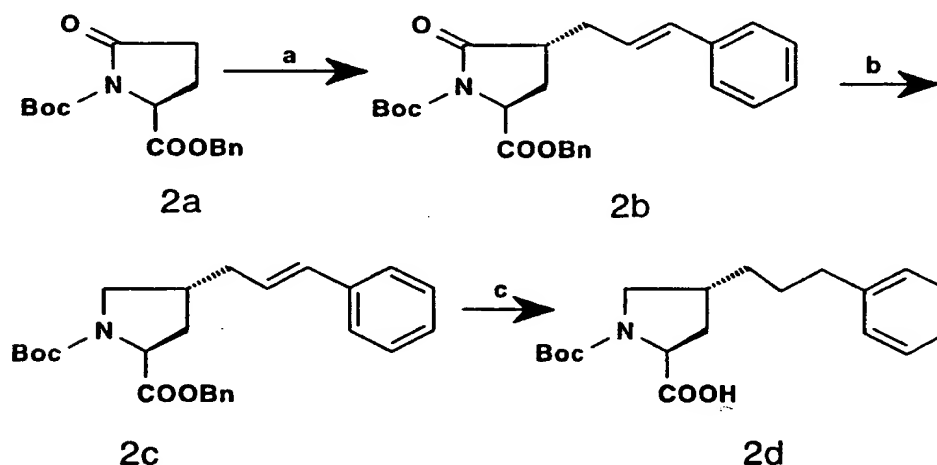
a) Synthesis of compound 2b:

To a solution of Boc-pyroglutamic acid benzyl ester
(**2a**) (prepared as described by A.L Johnson et al., J.
20   Med. Chem. (1985), **28**, 1596-1602) (500 mg, 1.57 mmol)
in THF (10 mL) at -78°C, was slowly added lithium
hexamethydisilylazide (1.72 mL, 1M solution in THF).
After stirring for 1 h at -78°C, cinnamyl bromide
(278 µL, 1.88 mmol) was added and the stirring
25   continued for an additional 2 h. The reaction

mixture was quenched with saturated ammonium chloride
solution and extracted with ethyl ether (3 x 20 mL).
The combined organic extracts were dried (MgSO$_4$),
filtered and concentrated. The residue was purified
5   by flash column chromatography (8:2 hexane:ethyl
acetate) to give compound **2b** as an off-white solid
(367 mg, 54% yield). ^{1}H NMR (CDCl$_3$): δ 7.35-7.19 (m,
10H), 6.43 (d, J=15 Hz, 1H), 6.11 (ddd, J=15, J'=J"=8
Hz, 1 H), 5.26 (d, J=16 Hz, 1H), 5.17 (d, J=16 Hz,
10   1H), 4.59 (dd, J=9.5, J'=2 Hz, 1 H), 2.83-2.70 (m,
2H), 2.41-2.34 (m, 1H), 2.22-2.16 (m, 1H), 2.10-2.02
(m, 1H) 1.42 (s, 9 H).

b) Synthesis of compound 2c:

15   At -78°C, lithium triethylborohydride (1M solution in
THF, 1.01 mL, 1.01 mmol) was added to a solution of
compound **2b** (367 mg, 0.843 mmol) in THF (5 mL), under
a nitrogen atmosphere. After 30 min, the reaction
mixture was quenched with saturated aqueous NaHCO$_3$ (2
20   mL) and warmed to 0°C. 30% H$_2$O$_2$ (5 drops) was added
and the mixture was stirred at 0°C for 20 min. The
organic volatiles were removed *in vacuo*, and the
aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 10 mL).
The combined organic extracts were dried (MgSO$_4$),
25   filtered and concentrated. To a cold (-78°C) solution
of the residue and triethylsilane (134 μL, 0.843
mmol) in CH$_2$Cl$_2$ (3 mL) boron trifluoride etherate (118
μL, 0.927 mmol) was added dropwise under an atmosphere
of nitrogen. After 30 min, additional triethylsilane
30   (134 μL) and boron trifluoride etherate (118 μL) were
added. After stirring for 2 h at -78°C, the reaction
mixture was quenched with saturated aqueous NaHCO$_3$ (2
mL) and extracted with DCM (3 x 10 mL). The combined
organic extracts were dried (MgSO$_4$), filtered and

concentrated. The crude product was purified by
flash column chromatography (8:2 hexane:ethyl
acetate) to give compound **2c** as a colorless oil (140
mg, 40% yield). ^{1}H NMR (CDCl$_3$) indicated the presence

5   of two rotamers: δ 7.34-7.22 (m, 10H), 6.38 (d,
J=15.5 Hz, 1H), 6.15-6.08 (m, 1H), 5.29-5.07 (m, 2H),
4.44 (d, J=7 Hz, 1/3H), 4.33 (d, J=7 Hz, 2/3H), 3.76
(dd, J=10.5, J'=8.5 Hz, 2/3H), 3.69 (dd, J=10.5,
J'=8.5 Hz, 1/3H), 3.13 (dd, J=9, J'=8.5 Hz, 2/3H),

10   3.05 (dd, J=9, J'=8.5 Hz, 1/3H), 2.47-2.40 (m, 1H),
2.35-2.22 (m, 2H) 2.15-1.85 (m, 2H), 1.45 (s, (3/9)
9H), 1.33 (s, (6/9) 9H).

c) Synthesis of compound 2d:

15   To a solution of compound **2c** (140 mg, 0.332 mmol) in
ethanol (4 mL) was added 10% palladium on charcoal
(30 mg). The mixture was stirred under an atmosphere
of hydrogen for 2 h. The catalyst was removed by
passing the mixture through a Millipore: Millex - HV

20   0.45 μm filter. The clear solution was concentrated
to give the desired compound **2d** as a colorless oil
(115 mg, quant. yield). ^{1}H NMR (DMSO-d$_6$) indicated
the presence of two rotamers: δ 7.28-7.14 (m, 5H),
4.33 (br.s, 1H), 4.06-4.10, (m, 1H), 3.56-3.42 (m,

25   3H), 2.89-2.79 (m, 1H),), 2.53-2.49 (m, 1H, under
DMSO-d$_6$), 2.24-2.10 (m, 1H), 2.03-1.93 (m, 1H), 1.87-
1.75 (m, 1H), 1.62-1.45 (m, 2H), 1.38 (s, (3/9) 9H),
1.33 (s, (6/9) 9H).

30   **Example 3**
Synthesis of Boc-4(R)-(naphthalen-1-ylmethoxy)
proline (3):

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(3)
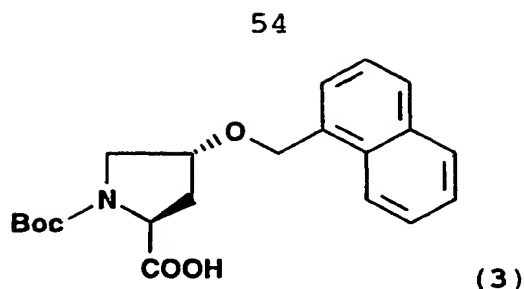
Commercially available Boc-4*(R)*-hydroxyproline (5.00
g, 21.6 mmol) was dissolved in THF (100 mL) and
5   cooled to 0°C. Sodium hydride (60% dispersion in oil,
1.85 g, 45.4 mmol) was added portionwise over 10
minutes and the suspension was stirred at RT for 1 h.
Then, 1-(bromomethyl)naphthalene (8.00 g, 36.2 mmol)
(prepared as described in E.A. Dixon et al. Can. J.
10   Chem., (1981), 59, 2629-2641) was added and the
mixture was heated at reflux for 18 h. The mixture
was poured into water (300 mL) and washed with
hexane. The aqueous layer was acidified with 10%
aqueous HCl and extracted twice with ethyl acetate.
15   The organic layers were combined and washed with
brine, dried (MgSO$_4$), filtered and concentrated. The
residue was purified by flash chromatography (49:49:2
hexane: ethyl acetate: acetic acid) to give the title
compound as a colorless oil (4.51 g, 56% yield). ^{1}H
20   NMR (DMSO-d$_6$) indicated the presence of two rotamers:
δ 8.05 (m, 1H), 7.94 (m, 1H), 7.29 (d, J=14 Hz, 1H),
7.55-7.45 (m, 4H), 4.96 (m, 2H), 4.26 (br. s, 1H),
4.12 (dd, J=J=8 Hz, 1H), 3.54-3.42 (m, 2H), 2.45-2.34
(m, 1H), 2.07-1.98 (m, 1H) 1.36 (s, (3/9) 9H), 1.34
25   (s, (6/9) 9H).

55

Example 4

Synthesis of Boc-4(R)-(8-quinoline-methyloxy) proline (4):
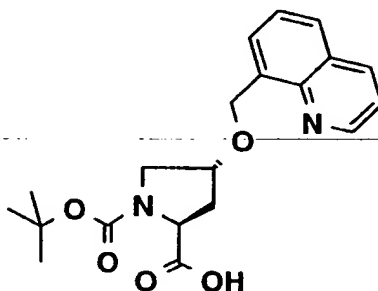
(4)

5   Boc-4(R)-hydroxyproline (1.96 g, 8.5 mmol) in anhydrous THF (20 mL) was added to a suspension of NaH (1.4 g, 60% in oil, 34 mmol) in THF (100 mL). This mixture was stirred 30 min before bromomethyl-8-quinoline from Example 1 (2.54 g, 11.44 mmol) was

10   added in THF (30 mL). The reaction mixture was heated at 70°C (5 h) before the excess NaH was destroyed carefully with wet THF. The reaction was concentrated *in vacuo* and the resulting material was dissolved in EtOAc and H_2O. The basic aqueous phase was separated

15   and acidified with 10% aqueous HCl to pH ~5 before being extracted with EtOAc (150 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated to give a brown oil. Purification by flash chromatography (eluent: 10% $MeOH/CHCl_3$) gave the

20   desired compound as a pale yellow solid (2.73 g, 86%). HPLC (97.5%); ^{1}H-NMR (DMSO-d_6) shows rotamer populations in a 6:4 ratio, δ 12-11.4 (bs, 1H), 8.92 (2 x d, J = 4.14 and 4.14 Hz, 1H), 8.38 (2 x d, J = 8.27 and 8.27 Hz, 1H), 7.91 (d, J = 7.94 Hz, 1H),

25   7.77 (d, J = 7.0 Hz, 1H), 7.63-7.54 (m, 2H), 5.14 (2 x s, 2H), 4.32-4.29 (m, 1H), 4.14-4.07 (m, 1H), 3.52-3.44 (m, 2H), 2.43-2.27 (m, 1H), 2.13-2.04 (m, 1H), 1.36 and 1.34 (2 x s, 9H).

Example 5

Preparation of Boc-4(R)-(7-chloroquinoline-4-oxo)proline (5):

(5)

5
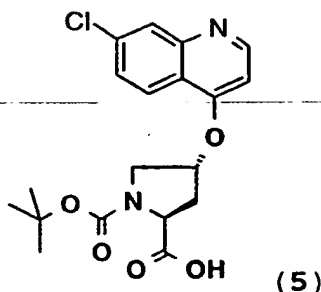
Commercially available Boc-4(S)-hydroxyproline methyl ester (500 mg, 2.04 mmol) and 7-chloro-4-hydroxyquinoline (440 mg, 2.45 mmol) were placed in dry THF (10 mL) at 0°C. Triphenylphosphine (641 mg,

10   2.95 mmol) was added, followed by slow addition of DIAD (426 mg, 2.45 mmol). The mixture was stirred at RT for 20 h. The reaction mixture was then concentrated, taken up in ethyl acetate and extracted three times with HCl 1N. The aqueous phase was

15   basified with Na_2CO_3 and extracted twice with ethyl acetate. The organic layers were combined, dried over $MgSO_4$, filtered and concentrated to give a yellow oil. The oil was purified by flash chromatography to give compound (5) methyl ester as

20   a white solid, 498 mg, 58% yield.

This methyl ester (400 mg, 0.986 mmol) was hydrolysed with 1M aqueous sodium hydroxide (1.7 mL, 1.7 mmol) in methanol (4 mL), at 0°C, for 3 h. The solution

25   was concentrated to remove the methanol and neutralised with 1M aqueous HCl. The suspension was concentrated to dryness and taken up in methanol (20 mL), the salts were filtered off and the filtrate concentrated to give the desired compound (5) as a

white solid, 387 mg, quant. yield.

^{1}H NMR (DMSO-d_6) (ca. 1:1 mixture of rotamers) δ 8.74 (d, J = 5 Hz, 1 H), 8.13-8.09 (m, 1 H), 7.99 and 7.98 (s, 1 H), 7.58 (d, J = 9 Hz, 1 H), 7.02 (d, J = 5 Hz, 1 H), 5.26-5.20 (m, 1 H), 4.10- 4.01 (m, 1 H), 3.81- 3.72 (m, 1 H), 3.59 (dd, J = 12, 10 Hz, 1 H), 2.41- 2.31 (m, 2 H), 1.34 and 1.31 (s, 9H).

Example 6

General procedure for coupling reactions done on solid support.

The synthesis was done on a parallel synthesizer model ACT396 from Advanced ChemTech® with the 96 well block. Typically, 24 peptides were synthesized in parallel using standard solid-phase techniques. The starting Fmoc-Nva-Wang resin and the 1-(Fmoc-amino)cyclopropane carboxylic acid-Wang resin were prepared by the DCC/DMAP coupling method (Atherton, E; Scheppard, R.C. *Solid Phase Peptide Synthesis, a Practical Approach*; IRL Press: Oxford 1989; pp 131-148). Other amino acid-Wang resins were obtained from commercial sources.

Each well was loaded with 100 mg of the starting resin (approximately 0.05 mmol). The resins were washed successively with 1.5 mL portions of NMP (1 X) and DMF (3 X). The Fmoc protecting group was removed by treatment with 1.5 mL of a 25% v/v solution of piperidine in DMF for 20 min. The resins were washed with 1.5 mL portions of DMF (4 X), MeOH (3 X) and DMF (3 X). The coupling was done in DMF (350 μL), using 400 μL (0.2 mmol) of a 0.5M solution of Fmoc-amino

acid/HOBt hydrate in DMF, 400 μL (0.4 mmol) of a 0.5M
solution of DIPEA in DMF and 400 μL (0.2 mmol) of a
0.5M solution of TBTU in DMF. After shaking for 1 h,
the wells were drained, the resins were washed with
5   1.5 mL of DMF and the coupling was repeated once more
under the same conditions. The resins were then
washed as described above and the cycle was repeated
with the next amino acid.

10   The capping groups were introduced in two ways:
1. In the form of a carboxylic acid using the
protocol described above (for example acetic acid)
or,
2. As an acylating agent such as an anhydride or an
15   acid chloride. The following example illustrates the
capping with succinic anhydride: After the Fmoc
deprotection and subsequent washing protocol, DMF was
added (350 μL), followed by 400 μL each of a DMF
solution of succinic anhydride (0.5 M, 0.2 mmol) and
20   DIPEA (1.0 M, 0.4 mmol). The resins were stirred for
2 h and a recoupling step was performed.

At the end of the synthesis the resin was washed with
1.5 mL portions of DCM (3 x), MeOH (3 x), DCM (3 x),
25   and were dried under vacuum for 2 h.

The cleavage from the resin and concomitant side
chain deprotection was effected by the addition of
1.5 mL of a mixture of TFA, H_2O, DTT and TIS (92.5:
30   2.5: 2.5: 2.5). After shaking for 2.5 h, the resin
was filtered and washed with 1.5 mL of DCM. The
filtrates were combined and concentrated by vacuum
centrifugation.

Each compound was purified by preparative reversed phase HPLC using a C18 column (22 mm by 500 mm). The product-containing fractions were identified by MALDI-TOF mass spectrometry, combined and

5   lyophilized.

Example 7

General procedure for coupling reactions done in solution (See also R. Knorr et al., Tetrahedron

10   **Letters, (1989), <u>30</u>, 1927.)**

The reactants, i.e. a free amine (1 eq.) (or its hydrochloride salt) and the free carboxylic acid (1 eq.) were dissolved in CH_2Cl_2, CH_3CN or DMF. Under a

15   nitrogen atmosphere, four equivalents of N-methylmorpholine and 1.05 equivalents of the coupling agent were added to the stirred solution. After 20 min, one equivalent of the second reactant, i.e. a free carboxylic acid was added. (Practical and

20   efficient coupling reagents for this purpose are (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate (HOBT) or preferably 2-(1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) or O-(7-azabenzotriazol-1-

25   yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (HATU). The reaction is monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc. The solution was washed

30   successively with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine. The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure. When the residue was purified, it was done by flash chromatography as defined above.

Example 8

Synthesis of segment: Ac-Chg-Chg-Pro (4(R)-naphthalen-1-ylmethoxy)-OH (8g)
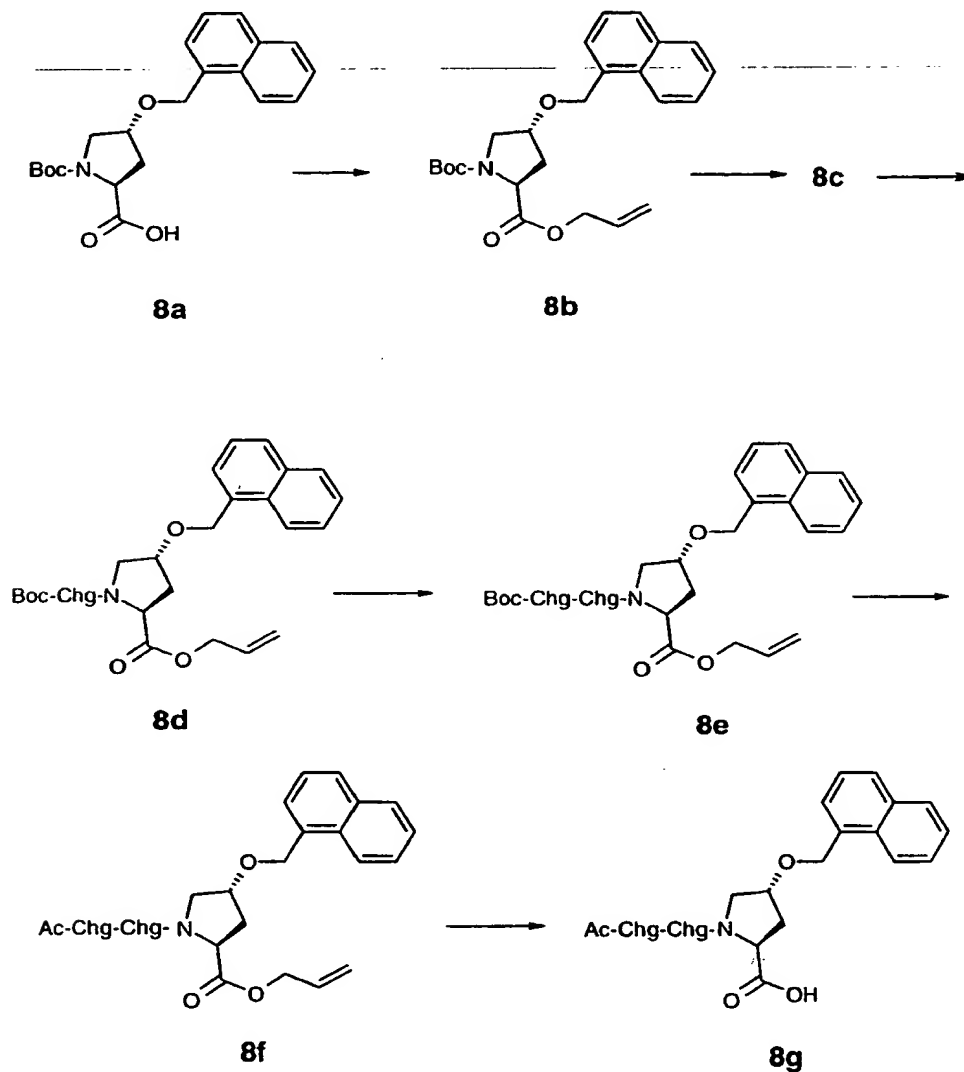
Compound **8a** (4.45g, 11.98 mmol) was dissolved in anhydrous CH₃CN (60 mL). DBU (2.2 mL, 14.38mmol) and allyl bromide (1.1 mL, 13.18 mmol) were added successively and the reaction mixture was stirred 24 h at RT. The mixture was concentrated, the resulting oil was diluted with EtOAc and water and successively washed with water (2x) and brine (1x). The EtOAc layer was dried (MgSO₄), filtered and evaporated to dryness. The yellow oil was purified by flash

chromatography (eluent:hexane:EtOAc;90:10 to 85:15) to provide the product **8b** as a yellow oil (2, 4.17g ; 85 % yield). MS (FAB) 412 MH$^+$

^{1}H NMR (CDCl$_3$) , mixture of rotamers ca.1:2 , δ (d, J= 8Hz, 1H), 7.87 (d, J= 8Hz, 1H), 7.82 (d, J= 8Hz, 1H), 7.55-7.41 (m, 4H), 5.95-5.85 (m, 1H), 5.34-5.21 (m, 2H), 5.03-4.88 (m, 2H), 4.70-4.56 (m, 2H), 4.48 & 4.39 (t, J= 8, 15Hz, 1H), 4.28-4.23 (m, 1H), 3.81-3.55 (m, 2H), 2.46-2.36 (m, 1H), 2.13-2.05 (m, 1H), 1.44 & 1.41 (s, 9H).

Compound **8b** (2.08 g , 5.05 mmol) was treated for 30 min at RT with 4N HCl / dioxane. Evaporation to dryness provided the corresponding amine-HCl as an oil. The amine-HCl **8c** was dissolved in anhydrous DCM (25 mL) , NMM (2.2 mL, 20.22 mmol), Boc-Chg-OH · H$_2$O (1.53 g, 5.56 mmol) and TBTU (1.95 g, 6.07 mmol) were added successively. The reaction mixture was stirred at RT overnight, then, diluted with EtOAc and successively washed with 10% aqueous citric acid (2x), saturated aqueous NaHCO$_3$ (2x), water (2x), and brine (1x). The EtOAc layer was dried (MgSO$_4$), filtered and evaporated to dryness to provide the crude product **8d** as a yellowish-white foam (ca 2.78g, 100% yield). MS (FAB) 551.4 MH$^+$. ^{1}H NMR (CDCl$_3$) δ 8.03(d, J= 8Hz, 1H), 7.86 (b d, J= 8.5Hz, 1H), 7.84 (d, J= 8Hz, 1H), 7.56-7.40 (m, 4H), 5.92-5.85 (m, 1H), 5.31 (dd, J= 1, 17Hz, 1H), 5.22 (dd, J= 1, 10Hz, 1H), 5.17 (d, J= 9Hz, 1H), 5.05 (d, J= 12Hz, 1H), 4.91 (d, J= 12Hz, 1H), 4.67-4.60 (m, 3H), 4.31-4.27 (m, 2H), 4.16 (b d, J= 11Hz, 1H), 3.71 (dd, J= 4, 11Hz, 1H), 2.47-2.41 (m, 1H), 2.08-1.99 (m,1H), 1.85-1.63 (m, 5H), 1.44-1.40 (m, 1H), 1.36 (s, 9H), 1.28-1.00 (m, 5H).

The crude dipeptide **8d** (ca. 5.05 mmol) was treated
with 4N HCl/dioxane (25 mL) as described for compound
8c. The crude hydrochloride salt was coupled to Boc-
Chg-OH · H_2O (1.53 g, 5.55 mmol) with NMM (2.22 mL,
20.22 mmol) and TBTU (1.95 g, 6.07 mmol) in DCM (25
mL) as described for compound **8d** to yield crude
tripeptide as a yellow-oil foam. The crude material
was purified by flash chromatography
(eluent:hexane:EtOAc;80:20 to 75:25) to provide the
tripeptide **8e** as a white foam (2.75 g; 79% yield over
2 steps). MS (FAB) 690.5 MH^+. ^1H NMR ($CDCl_3$), mainly
one rotamer, δ 8.06 (d, J= 8Hz, 1H), 7.87 (b d, J=
8.5Hz, 1H), 7.82 (d, J= 8Hz, 1H), 7.57-7.40 (m, 4H),
6.41 (d, J= 8.5Hz, 1H), 5.92-5.84 (m, 1H), 5.31 (dd,
J= 1, 17Hz, 1H), 5.23 (dd, J= 1, 10.5Hz, 1H), 5.04
(d, J= 12Hz, 1H), 4.98 (b d, J= 7Hz, 1H), 4.93 (d,
J=12Hz, 1H), 4.63-4.58 (m, 4H), 4.29-4.25 (m, 1H),
4.10-4.07 (m, 1H), 3.90-3.84 (m, 1H), 3.72 (dd, J= 4,
11Hz, 1H), 2.48-2.40 (m, 1H), 2.07-1.99 (m, 1H),
1.83-1.55 (m, 12H), 1.43 (s, 9H), 1.23-0.89 (m, 10H).

The tripeptide **8e** (2.75 g, 3.99 mmol) was treated
with 4N HCl/dioxane (20 mL) as described for compound
8c. The crude hydrochloride salt was dissolved in
anhydrous DCM (20 mL). NMM (1.75 mL, 15.94 mmol) and
acetic anhydride (752 μL, 7.97 mmol) were added
successively. The reaction mixture was stirred
overnight at RT, then diluted with EtOAc. The organic
layer was washed successively with 10% aqueous
citric acid (2x), saturated aqueous $NaHCO_3$ (2x),
water (2x) and brine (1x), dried ($MgSO_4$), filtered,
and evaporated to dryness to provide the crude
tripeptide **8f** as a white foam (2.48 g, 98% yield).

MS (FAB) 632.4 MH^{+1}. ^{1}H NMR (CDCl$_3$), mainly one
rotamer, δ 8.06(b d, J= 8Hz, 1H), 7.87 (b d, J= 8Hz,
1H), 7.83 (d, J= 8Hz, 1H), 7.58-7.40 (m, 4H), 6.36
(d, J= 9Hz, 1H), 6.01 (d, J= 9Hz, 1H), 5.94-5.83 (m,

5   1H), 5.34-5.28 (m, 1H), 5.25-5.21 (m, 1H), 5.05 (d,
J= 12Hz, 1H), 4.94 (d, J= 12Hz, 1H), 4.64-4.57 (m,
4H), 4.30-4.23 (m, 2H), 4.12-4.08 (m, 1H), 3.73 (dd,
J= 4, 11Hz, 1H), 2.49-2.42 (m, 1H), 2.08-2.01 (m,
1H), 1.99 (s, 3H), 1.85-1.53 (m, 11H), 1.25-0.88 (m,

10   11H).

The crude tripeptide **8f** (2.48 g, 3.93 mmol)was
dissolved in an anhydrous mixture of CH$_3$CN : DCM (20
mL). Triphenylphosphine (53.5 mg, 0.200 mmol) and

15   tetrakis(triphenylphosphine)-palladium (0) catalyst
(117.9 mg, 0.102 mmol)were added successively,
followed by pyrrolidine (353.9 µL, 4.24 mmol). The
reaction mixture was stirred at RT for 18 h.
Thereafter, the solvent was evaporated. The residue

20   was dissolved in EtOAc and 10% aqueous citric acid ,
then, further washes twice more with 10% aqueous
citric acid, water (2x), and brine (1x). The organic
layer was dried (MgSO$_4$), filtered and evaporated. The
crude product was triturated in Et$_2$O: DCM (85:15) to

25   provide after filtration the tripeptide **8g** as a white
solid (2.09 g, 90% yield). MS (FAB) 592.4 MH$^+$
614.3 (M+Na) $^+$. ^{1}H NMR (CDCl$_3$), mainly one rotamer, δ
8.08 (d, J= 8Hz, 1H), 7.93 (b d, J= 9Hz, 1H), 7.88
(b d, J= 8Hz, 1H), 7.82 (d, J= 8Hz, 1H), 7.57-7.41

30   (m, 4H), 6.47 (d, J= 8.5Hz, 1H), 5.05 (d, J= 12.5Hz,
1H), 4.94 (d, J= 12.5Hz, 1H), 4.73 (t, J= 9.5, 19Hz,
1H), 4.44-4.35 (m, 2H), 4.26 (b s, 1H), 4.19 (d, J=
11.5Hz, 1H), 3.75 (dd, J= 4, 11Hz, 1H), 2.47 (b dd,

64

J= 7.5, 13.5Hz, 1H), 2.20-2.11 (m, 1H), 2.04 (s, 3H),

1.88-1.41 (m, 11H), 1.30-0.80 (11H).

Example 9

5   **Synthesis of segment: Ac-Chg-Val-Pro(4(R)-naphthalen-**

1-ylmethoxy)-OH (9e)

9a ≡ 8b   9b

9c   9d

9e
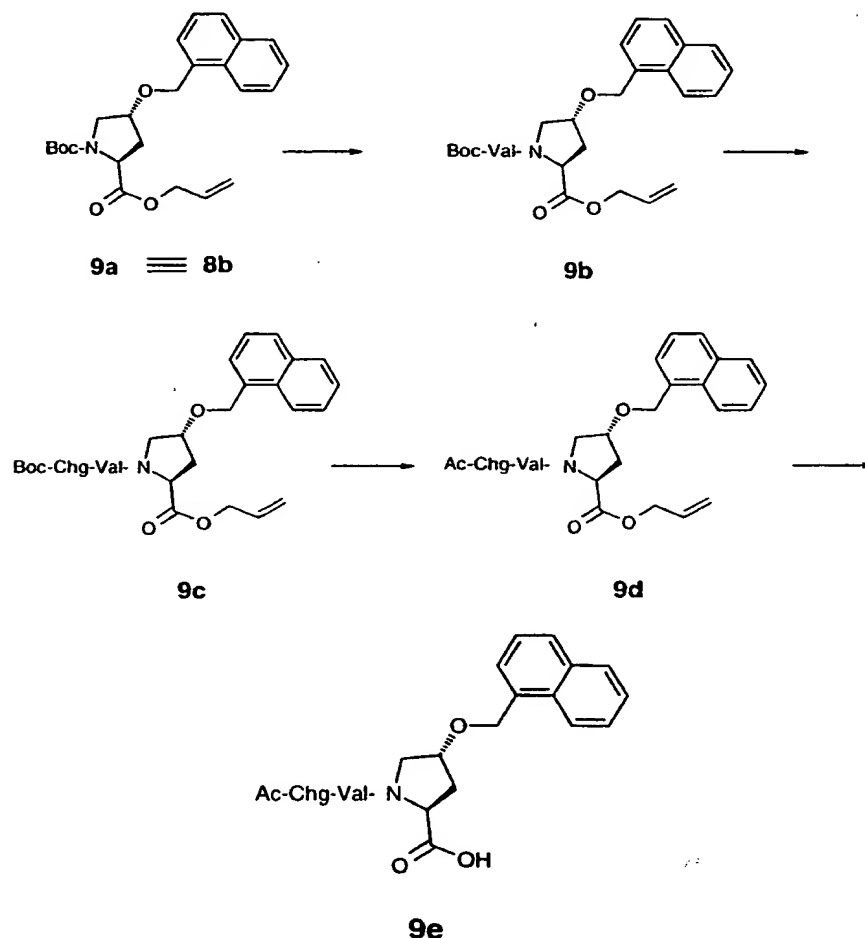
Compound **9a** (2.89 g, 7.02 mmol) was treated with 4N

10   HCl/dioxane (30 mL) as described for compound **8c**. The

crude hydrochloride salt was coupled to Boc-Val-OH

(1.53 g, 7.73 mmol) with NMM (3.1 mL, 28.09 mmol) and

TBTU (2.71 g, 8.43 mmol) in DCM (35 mL) for 3.5 h as

described for compound **3** to provide the crude

15   dipeptide **9b** as an ivory oil-foam (ca.3.60 g, 100%

yield). MS (FAB) 509.3 MH⁻ 511.3 MH⁺ 533.2

(M+Na)⁺. ¹H NMR (CDCl₃) δ 8.04 (b d, J= 8Hz, 1H),

7.87 (b d, J= 7Hz, 1H), 7.82 (d, J= 8Hz, 1H), 7.56-
7.40 (m, 4H), 5.93-5.85 (m, 1H), 5.34-5.28 (m, 1H),
5.24-5.19 (m, 2H), 5.04 (d, J= 12Hz, 1H), 4.92 (d, J=
12Hz, 1H), 4.67-4.60 (m, 3H), 4.31-4.26 (m, 2H),
4.11-4.09 (m, 1H), 3.72 (dd, J= 4, 11Hz, 1H), 2.48-
2.41 (m, 1H), 2.07-1.99 (m, 1H), 1.44-1.36 (m, 1H),
1.37 (s, 9H), 1.01 (d, J= 7Hz, 3H), 0.93 (d, J= 7Hz,
3H).

The crude dipeptide **9b** (ca. 7.02 mmol) was treated
with 4N HCl/dioxane (30 mL) as described for compound
7c. The crude hydrochloride salt was coupled to Boc-
Chg-OH · H_2O (2.13 g, 7.73 mmol) with NMM (3.1 mL,
28.09 mmol) and TBTU (2.71 g, 8.43 mmol) in CH_2Cl_2
(35 mL) as described for compound 3 to provide the
crude tripeptide **9c** as an ivory foam (ca.4.6 g, 100%
yield). MS (FAB) 648.5 MH^- 672.4 $(M+Na)^+$. ^1H
NMR ($CDCl_3$) δ 8.06 (b d, J=8Hz, 1H), 7.87 (b d, J=
7.5 Hz, 1H), 7.82 (b d , J= 8Hz, 1H), 7.57-7.40 (m,
4H), 6.46 (b d, J= 8.5Hz, 1H), 5.94-5.84 (m, 1H),
5.31 (dd, J= 1, 17Hz, 1H), 5.23 (dd, J= 1, 10.5Hz,
1H), 5.03 (d, J= 12Hz, 1H), 5.00-4.97 (m, 1H), 4.93
(d, J=, 12Hz, 1H), 4.63-4.59 (m, 4H), 4.29-4.27 (m,
1H), 4.10-4.07 (m, 1H), 3.92-3.86 (m, 1H), 3.72 (dd,
J= 5, 11Hz, 1H), 2.48-2.41 (m, 1H), 2.10-1.99 (m,
1H), 1.76-1.57 (m, 6H), 1.43 (s, 9H), 1.20-0.92 (m,
6H), 1.00 (d, J= 7Hz, 3H), 0.93 (d, J= 7Hz, 3H).

The crude tripeptide **9c** (ca.7.02 mmol) was treated
with 4N HCl/dioxane (30 mL) as described for compound
8c. The crude hydrochloride salt was further treated
with acetic anhydride (1.33 mL, 14.05 mmol) and NMM
(3.1 mL, 28.09 mmol) in CH_2Cl_2 (35 mL) as described
for compound **8f**. The crude product was flash

purified (eluent:hexane:EtOAc;30:70) to provide the
acetylated protected tripeptide **9d** as a white foam
(3.39 g, 81% yield over 3 steps).   MS (FAB)   590.3
MH^- 592.4 MH^+ 614.4 $(M+Na)^+$

5   ^1H NMR ($CDCl_3$), mainly one rotamer, δ 8.06 (d, J= 8Hz,
1H), 7.88 (b d, J= 8Hz, 1H), 7.83 (d, J= 8Hz, 1H),
7.58-7.41 (m, 4H), 6.37 (d, J= 9Hz, 1H), 5.97 (d, J=
8.5 Hz, 1H), 5.94-5.84 (m, 1H), 5.31 (dd, J= 1, 17Hz,
1H), 5.24 (dd, J= 1, 10.5 Hz, 1H), 5.05 (d, J= 12Hz,
10   1H), 4.94 (d, J= 12Hz, 1H), 4.66-4.57 (m, 4H), 4.31-
4.22 (m, 2H), 4.11-4.05 (m, 1H), 3.73 (dd, J= 4.5,
11Hz, 1H), 2.50-2.43 (m, 1H), 2.09-2.01 (m, 2H), 2.00
(s, 3H), 1.68-1.55 (m, 5H), 1.15-0.89 (m, 6H), 0.99
(d, J= 7Hz, 3H), 0.91 (d, J= 7Hz, 3H).

15

The acetylated tripeptide **9d** (3.39 g, 5.73 mmol) was
deprotected by tetrakis(triphenylphosphine)-
palladium (0) catalyst (172.1 mg, 0.149 mmol) with
triphenylphosphine (78.1 mg, 0.298 mmol) and
20   pyrrolidine (516 μL, 6.19 mmol) in a 1:1 mixture of
anhydrous CH_3CN : DCM (30 mL) as described for
compound **8g**. The crude light yellow foam product was
triturated in Et_2O : DCM (85:15)to provide after
filtration the tripeptide **9e** as an off-white solid
25   (3.0 g; 95% yield).   MS (FAB) 550.3 MH^-

^1H NMR ($CDCl_3$) δ 8.08 (d, J= 8Hz, 1H), 8.04 (b d, J=
9Hz, 1H), 7.88 (b d, J= 7.5Hz, 1H), 7.82 (d, J= 8Hz,
1H), 7.58-7.37 (m, 5H), 5.05 (d, J= 12Hz, 1H), 4.94
(d, J= 12Hz, 1H), 4.61 (t, J= 9.5, 19.5Hz, 1H), 4.46-
30   4.37 (m, 2H), 4.27 (b s, 1H), 4.17 (d, J= 11Hz, 1H),
3.74 (dd, J= 4, 11Hz, 1H), 2.49 (b dd, J= 7.5, 13Hz,
1H), 2.17-2.09 (m, 1H), 2.04 (s, 3H), 2.03-1.94 (m,
1H), 1.79 (b d, J= 12.5Hz, 1H), 1.62-1.43 (m, 5H),

67

1.08-0.85 (m, 5H), 1.00 (d, J= 7Hz, 3H), 0.90 (d, J= 7Hz, 3H)..

Example 10

5 **Synthesis of pentapeptide 10h:**

The synthesis was done as follows:
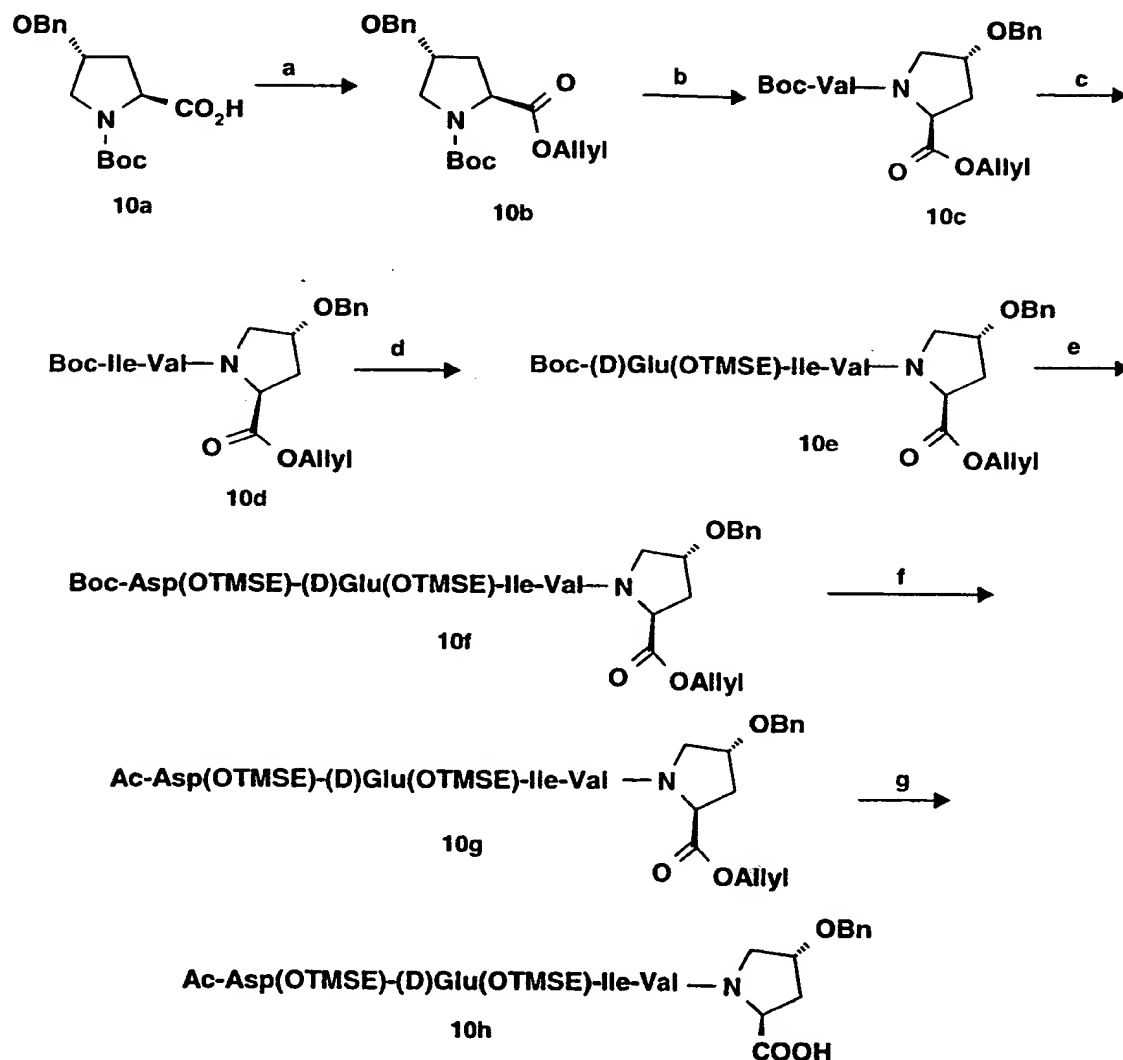
10 **a) Synthesis of compound 10b:**

To a solution of commercially available Boc-4(R)-benzyloxyproline (**10a**) (20.0 g, 62.2 mmol) in acetonitrile (250 mL) at 0°C were successively added DBU (10.3 mL, 68.87 mmol) and allyl bromide (6.0 mL,

15 69.3 mmol). The reaction mixture was stirred

68

overnight at RT, then the acetonitrile was evaporated
and the residue dissolved in EtOAc. The mixture was
sequentially washed with 10% aqueous citric acid
(2x), water, saturated aqueous $NaHCO_3$, water (2x) and
5   brine. The EtOAc solution was dried ($MgSO_4$), filtered
and concentrated to afford the desired ester **10b**
(21.84 g, 60.42 mmol, 97% yield) as a colourless oil.

b) Synthesis of compound 10c:

10   Allyl ester **10b** (21.84 g, 60.42 mmol) was treated
with a 4 N HCl solution in dioxane (453 mL, 1812.0
mmol) for 30 min before being concentrated *in vacuo*.
The amine hydrochloride was subjected to the reaction
conditions described in Example 3: The crude
15   hydrochloride salt was combined with Boc-Val-OH
(13.13 g, 60.43 mmol), NMM (26.6 mL, 241.93 mmol),
and TBTU (23.3 g, 72.56 mmol) in CH_2Cl_2 (300 mL). The
reaction mixture was stirred for 16 h at RT and then
concentrated *in vacuo*. The residue was dissolved in
20   EtOAc and washed sequentially with 10% aqueous citric
acid (2x), water, saturated aqueous $NaHCO_3$ (2x),
water (2x), and brine. The organic layer was dried
($MgSO_4$), filtered and concentrated to afford the
crude dipeptide **10c** (30.23 g). MS (FAB) 461 (MH^+).
25   ^{1}H-NMR ($CDCl_3$) δ 7.36-7.28 (m, 5H), 5.91-5.84 (m, 1H),
5.35-5.21 (m, 2H), 5.19 (bs, 1H), 4.66-4.62 (m, 3H),
4.56 (d, J = 11.8 Hz, 1H), 4.49 (d, J= 11.4 Hz, 1H),
4.28-4.24 (m, 2H), 4.04 (bd, J = 11.1 Hz, 1H), 3.70
(dd, J = 4.5, 11.1 Hz, 1H), 2.25-2.42 (m, 1H), 2.08-
30   1.98 (m, 1H), 1.45-1.40 (m, 1H), 1.41 (s, 9H), 1.01
(d, J = 6.7 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H).

c) Synthesis of compound 10d:

The crude dipeptide **10c** (ca. 60 mmol) was treated
with a 4 N HCl in dioxane solution (453 mL). The
crude hydrochloride salt was combined with Boc-Ile-OH
5   (14.0 g, 60.5 mmol), TBTU (23.3g, 72.6 mmol) and NMM
       (26.6 mL, 241.9 mmol, 4.0 eq.) in CH_2Cl_2 (300 mL) as
described for compound **10c** to afford the crude
tripeptide **10d**. Purification by flash chromatography
(using a mixture of 30% EtOAc in hexane) gave the
10   desired compound **10d** (25.61 g, 44.64 mmol, 72% yield
from compound **10a**). MS (FAB) 574 (MH$^+$); ^{1}H-NMR ($CDCl_3$)
δ 7.37-7.28 (m, 5H), 6.44 (d, J = 8.6 Hz, 1H), 5.95-
5.84 (m, 1H), 5.35-5.23 (m, 2H), 5.99(d, J = 8.4 Hz,
1H), 4.65-4.47 (m, 3H), 4.56 (d, J = 11.8 Hz, 1H),
15   4.49 (d, J = 12.1 Hz, 1H), 4.27-4.21 (m, 1H), 4.03
(bd, J = 10.8 Hz, 1H), 3.97-3.88 (m, 1H), 3.71 (dd, J
= 4.1 Hz, J = 10.8 Hz, 1H), 2.49-2.41 (m, 1H), 2.12-
2.00 (m, 2H), 1.85-1.74 (m, 1H), 1.55-1.40 (m, 2H),
1.43 (s, 9H), 1.16-1.04 (m, 1H), 1.00 (d, J = 6.7 Hz,
20   3H), 0.93 (d, J = 6.7 Hz, 3H), 0.89-0.82 (m, 6H).

d) Synthesis of compound 10e:

Tripeptide **10d** (25.60 g; 44.62 mmol) was treated with
a 4 N HCl in dioxane solution (30 min) before being
25   concentrated *in vacuo* to give 22.64 g of the
hydrochloride salt. The hydrochloride salt (14.97 g,
29.35 mmol) was combined with Boc-(D)Glu(OTMSE)-OH
(10.2 g, 29.35 mmol), TBTU (11.30 g, 35.23 mmol) and
NMM (11.30 g, 35.23 mmol) in CH_2Cl_2 (150 mL) as
30   described for compound **10c**. Compound **10e** was
obtained as an off-white foam (23.6 g). MS (FAB)
803.5 (MH$^+$); ^{1}H-NMR ($CDCl_3$) δ 7.34-7.28 (m, 5H), 6.74
(d, J = 8.26 Hz, 1H), 6.49 (d, J = 8.90 Hz, 1H),
5.93-5.86 (m, 1H), 5.44-5.36 (m, 1H), 5.35-5.22 (m,

2H), 4.64-4.48 (m, 6H), 4.27-4.15 (m, 4H), 4.02 (d,
J = 11.13 Hz, 1H), 3.71 (dd, J = 11.13, 4.45 Hz, 2H),
2.49-2.41(m, 2H), 2.40-2.34 (m, 1H), 2.18-2.00 (m,
3H), 1.96-1.71 (m, 2H), 1.50-1.40 (m, 1H), 1.43 (s,

5   9H), 1.15-1.04 (m, 1H), 1.02-0.95 (m, 1H), 0.97 (d, J
= 8.27 Hz, 3H), 0.90 (d, J = 7.00 Hz, 3H), 0.87-0.82
(m, 1H), 0.84 (d, J = 6.67 Hz, 6H), 0.04 (s, 9H).

e) Synthesis of compound 10f:

10   The crude tetrapeptide **10e** (ca. 28.91 mmol) was
treated with a 4 N HCl in dioxane solution for 30 min
(150 mL). The crude hydrochloride salt was combined
with Boc-Asp(OTMSE)-OH (10.15 g, 30.44 mmol), NMM
(12.7 mL, 115.6 mmol), and TBTU (11.14 g, 34.70 mmol)

15   in CH_2Cl_2 (150 mL) as described for compound **10c**.
Pentapeptide **10f** was obtained as a light yellow foam
(ca. 29.5 g). MS (FAB) 1018 (MH^+); ^{1}H-NMR ($CDCl_3$) δ
7.36-7.28 (m, 6H), 6.78 (d, J = 8.90 Hz, 1H), 6.72
(d, J = 8.58 Hz, 1H), 6.22 (d, J = 7.94 Hz, 1H),

20   5.93-5.83 (m, 1H), 5.33-5.21 (m, 2H), 4.62-4.57 (m,
6H), 4.49-4.36 (m, 2H), 4.30 (dd, J = 8.90, 6.35 Hz,
1H), 4.23-4.14 (m, 5H), 3.72 (dd, J = 11.13, 4.77 Hz,
2H), 2.89 (dd, J = 16.85, 6.36 Hz, 1H), 2.79 (dd, J =
16.85, 6.36 Hz, 1H), 2.48-2.27 (m, 3H), 2.21-1.94 (m,

25   5H), 1.46-1.42 (m, 1H), 1.45 (s, 9H) , 1.17-1.07 (m,
1H), 1.00-0.86 (m, 4H), 0.99 (d, J = 6.68 Hz, 3H),
0.93 (d, J = 6.68 Hz, 3H), 0.90 (d, J = 6.67 Hz,
6H), 0.04 (s, 9H), 0.02 (s, 9H).

30   **f) Synthesis of compound 10g:**
The Boc-pentapeptide **10f** (ca. 28.91 mmol) was treated
with a 4 N HCl in dioxane solution (150 mL) for 30
min before being concentrated *in vacuo.* The crude
hydrochloride salt was dissolved in anhydrous DMF

(150 mL) followed by the successive addition of
pyridine (51.4 mL, 636.2 mmol) and acetic anhydride
(51.6 mL, 546.5 mmol). The reaction mixture was
stirred overnight at RT then poured into brine and

5   extracted with EtOAc (3x). The combined organic
extracts were washed sequentially with 10% aqueous
citric acid (2x), saturated $NaHCO_3$ (2x), water (2x),
and brine (1x). The organic layer was dried ($MgSO_4$),
filtered and evaporated to dryness. The oil/foam

10   residue was purified by flash chromatography (eluent,
hexane:EtOAc; 4:6) to provide the acetylated
pentapeptide **10g** as a white amorphous solid (17.56 g,
63% yield from compound **10d**). MS (FAB) 960.7 (MH^+)
982.9 (MNa^+); ^{1}H-NMR ($CDCl_3$) δ 7.72 (d, J = 9.22 Hz,

15   1H), 7.35-7.28 (m, 6H), 7.12 (d, J = 9.54 Hz, 1H) ,
6.63 (d , J = 8.26 Hz, 1H) , 5.91-5.81 (m, 1H), 5.32-
5.22 (m, 2H), 5.20-4.96 (m, 1H), 4.68-4.54 (m, 5H),
4.49-4.36 (m, 3H), 4.28-4.20 (m, 3H), 4.19-4.12 (m,
2H), 3.74 (dd , J = 11.76, 5.40 Hz , 2H), 2.93 (dd,

20   J = 17.48, 4.45 Hz, 1H), 2.81 (dd, J = 17.49, 6.36
Hz, 1H), 2.47-2.39 (m, 2H), 2.33-2.24 (m, 1H), 2.14-
1.95 (m, 5H), 2.03 (s, 3H), 1.52-1.42 (m, 1H), 1.17-
1.07 (m, 1H), 1.02-0.88 (m, 16H), 0.04 (s, 9H), 0.03
(s, 9H).

25

g) Synthesis of compound 10h:
The pentapeptide **10g** (16.01 g, 16.67 mmol) was
suspended in anhydrous acetonitrile (100 mL) and
treated successively with triphenylphosphine (227.4

30   mg, 0.87 mmol) and tetrakis(triphenylphosphine)-
palladium (0) catalyst (501 mg, 0.433mmol) followed
by pyrrolidine (1.5 mL, 18.01 mmol). The reaction
mixture was mechanically stirred for 4 h at RT and
then concentrated *in vacuo*. The residue was dissolved

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in EtOAc and washed sequentially with 10% aqueous
citric acid (3x), water (3x), and brine (1x). The
organic phase was dried (MgSO$_4$), filtered and
evaporated to dryness. The crude product was purified
5   by flash chromatography (eluent, 1 % HOAc, 2.3 % MeOH
in CHCl$_3$) to provide the pentapeptide **10h** as a white
amorphous solid (11.45 g, 75% yield). MS (FAB) 920
(MH$^+$), 942 (MNa$^+$), 958.6 (M+K); ^{1}H-NMR (CDCl$_3$) δ
7.53(d, J = 8.90 Hz, 1H), 7.40 (d, J = 7.32 Hz, 1H),
10 7.35-7.28 (m, 5H), 7.22-7.17 (m, 1H), 6.83 (d, J =
7.31 Hz, 1H) 5.00-4.94 (m, 1H), 4.64-4.61 (m, 2H),
4.53-4.44 (m, 3H), 4.35 (dd, J = 8.26, 6.04 Hz, 1H),
4.28-4.24 (m, 1H), 4.18-4.09 (m, 5H), 3.72 (dd, J =
10.81, 4.14 Hz, 1H), 2.87-2.84 (m , 2H), 2.47-2.41
15 (m, 2H), 2.34-2.24 (m, 1H), 2.16-1.97 (m, 5H), 2.06
(s, 3H), 1.52-1.42 (m, 1H), 1.17-1.08 (m, 1H), 1.01-
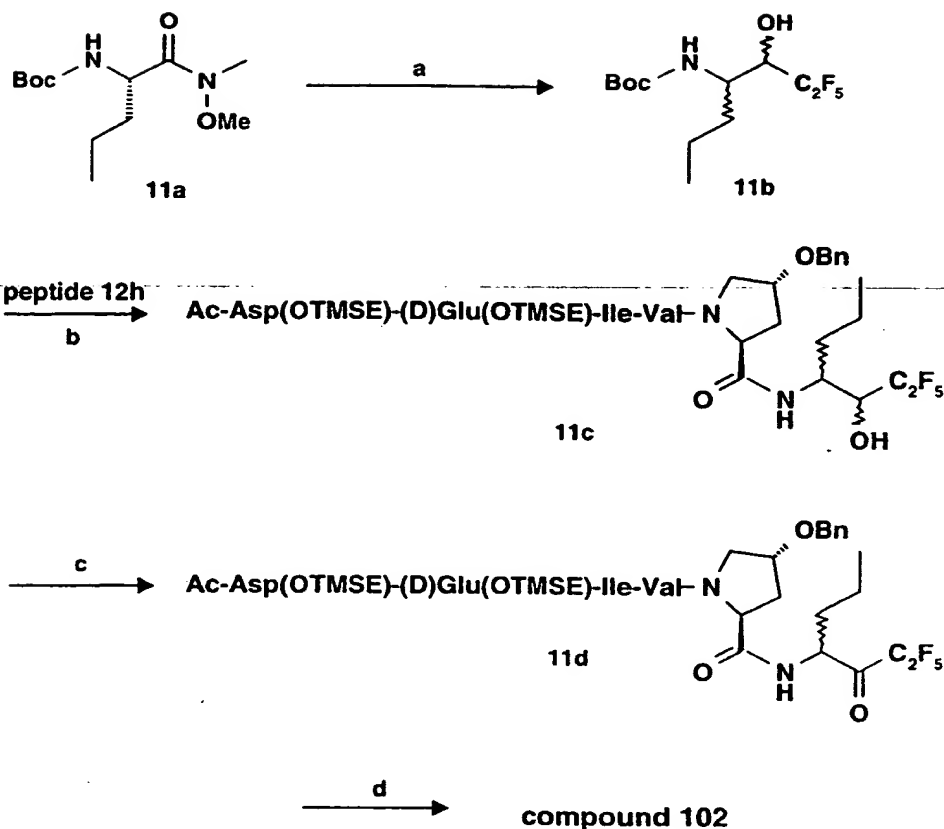0.84 (m, 16H), 0.04 (s, 9H), 0.03 (s, 9H).

Example 11
20 **Synthesis of compound 102 (Table 1)**
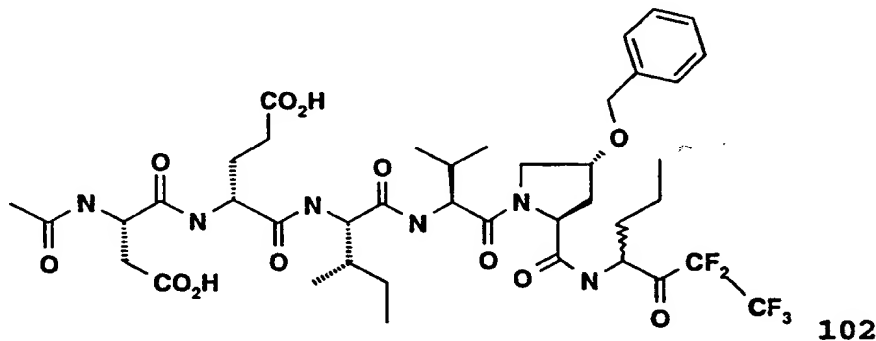
The synthesis was done as shown below:

73

a) Synthesis of compound 11b:

A cold (-78°C) solution of commercially available
5   pentafluoroethyl iodide (6.4 g, 26.02 mmol) in dry
     ether (16.7 mL) was slowly canulated (10 min) into a
     -78°C solution of MeLi.LiBr in ether (14.0 mL of a
     1.5 M solution in ether, 21.0 mmol). After an
     additional 10 min at -78°C Weinreb amide **11a**
10   (prepared from commercially available Boc-Nva-OH
     according to the procedure of Castro, B. et al.
     Synthesis, (1983), 676-678.)(2.02 g, 7.76 mmol) in
     ether (5 mL) was added. The reaction mixture was
     stirred for 1 hr at -78°C and then at -40°C for 15
15   min. A saturated aqueous solution of NH₄Cl was then
     added. The mixture was extracted 3 times with EtOAc
     and the combined organic extract was washed with

brine. The EtOAc solution was finally dried over
MgSO$_4$, filtered and concentrated.

The crude pentafluoroethyl ketone was dissolved in a
mixture of THF (38 mL) and MeOH (9.5 mL) and the

5   resulting solution was cooled to 0°C for the addition
of sodium borohydride (323 mg, 8.54 mmol, 1.1 eq.).
After 15 min, ether was added and the mixture was
washed with a 10% aqueous citric acid solution. The
aqueous layer was extracted 3 times with ether and

10   the combined organic layer was successively washed
with a saturated aqueous NaHCO$_3$ solution (2X), water
and brine. The ether solution was dried (Na$_2$SO$_4$),
filtered and concentrated. The residue was flash
chromatographed using a mixture of EtOAc (15%) and

15   hexane (85%) to afford the desired alcohols (1.30 g,
4.05 mmol, 52 % yield from the amide **11a**). ^{1}H NMR
(CDCl$_3$)(mixture of 2 diastereomers in a 1:1 ratio) δ
4.75 (bs, 1/2H), 4.54 (bs, 1/2H), 4.21-4.13 (m, 1H),
3.92 (dd, J= 6.0 Hz, J'= 14.6 Hz, 1H), 1.65-1.29 (m,

20   4H), 1.45 (m, 9H), 0.98-0.93 (m, 3H).

b) Synthesis of compound 11c:

Compound **11b** (96 mg, 0.30 mmol) was treated with a 4
N HCl solution in dioxane for 30 min before being

25   concentrated *in vacuo*. The crude hydrochloride salt
was dissolved in dry DMF (1 mL). The pentapeptide **10h**
(204 mg, 0.22 mmol), NMM (0.1 mL, 0.91 mmol, 4
eq.)and TBTU (85 mg, 0.26 mmol, 1.2 eq.) were then
successively added. The reaction mixture was stirred

30   overnight at RT , then poured into brine and
extracted 3 times with EtOAc. The combined organic
extract was successively washed with a 10% aqueous
citric acid solution (2x), water, saturated aqueous
NaHCO$_3$ solution, water (2x) and brine. The EtOAc

solution was dried (MgSO$_4$), filtered and
concentrated. The residue was purified by flash
chromatography using a mixture of EtOAc (75 to 80 %)
and hexane (25 to 20%) to afford the desired compound

5   **11c** (154 mg, 0.137 mmol, 62 % yield). MS (FAB) 1123
(MH$^+$).

c) Synthesis of compound 11d:

To the mixture of alcohols **11c** (52 mg, 0.047 mmol)

10 in a solution of DMSO (0.5 mL) and toluene (0.5 mL)
were successively added dichloroacetic acid (11.5 mL,
0.139 mmol) and EDAC (89 mg, 0.464 mmol). The
reaction mixture was stirred overnight at RT, poured
into brine (30 mL) and extracted with EtOAc (3x 15

15 mL). The combined organic extract was successively
washed with saturated aqueous NaHCO$_3$, water (2x) and
brine. The EtOAc solution was dried (MgSO$_4$), filtered
and concentrated. The residue was purified by flash
chromatography using a mixture of EtOAc (75 to 80%)

20 and hexane (25 to 20%) to afford the desired ketone
11d (37 mg, 0.032 mmol, 70% yield). MS (FAB) 1121
(MH$^+$).
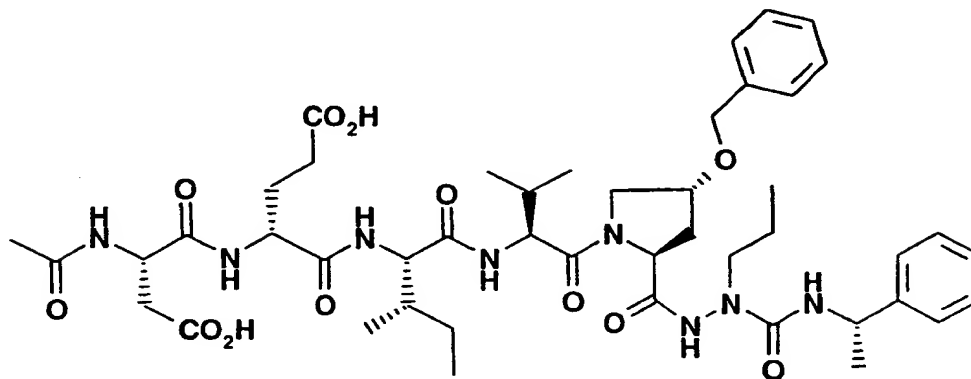
d) Synthesis of compound 102:

25 The mixture of ketone **11d** (37 mg, 0.032 mmol) was
dissolved in TFA (1 mL) and the resulting solution
was stirred for 1 h at RT. After removal of the
volatiles under vacuum, the desired peptide was
obtained (29 mg, 0.031 mmol, 97%). MS (FAB) 921

30 (MH$^+$), 943 (MNa$^+$). ^{1}H NMR (CDCl$_3$)(mixture of 2
diastereomers in a 1.35:1 ratio) d 8.14 (d, J= 7.6
Hz, 1H), 8.07-8.02 (m, 2H), 7.80-7.78 (m, 1H), 7.35-
7.26 (m,5H), 4.77-4.56 (m, 1H), 4.56-4.38 (m, 5H),
4.34-4.09 (m, 5H), 2.68-2.59 (m, 2H), 2.26-2.15 (m,

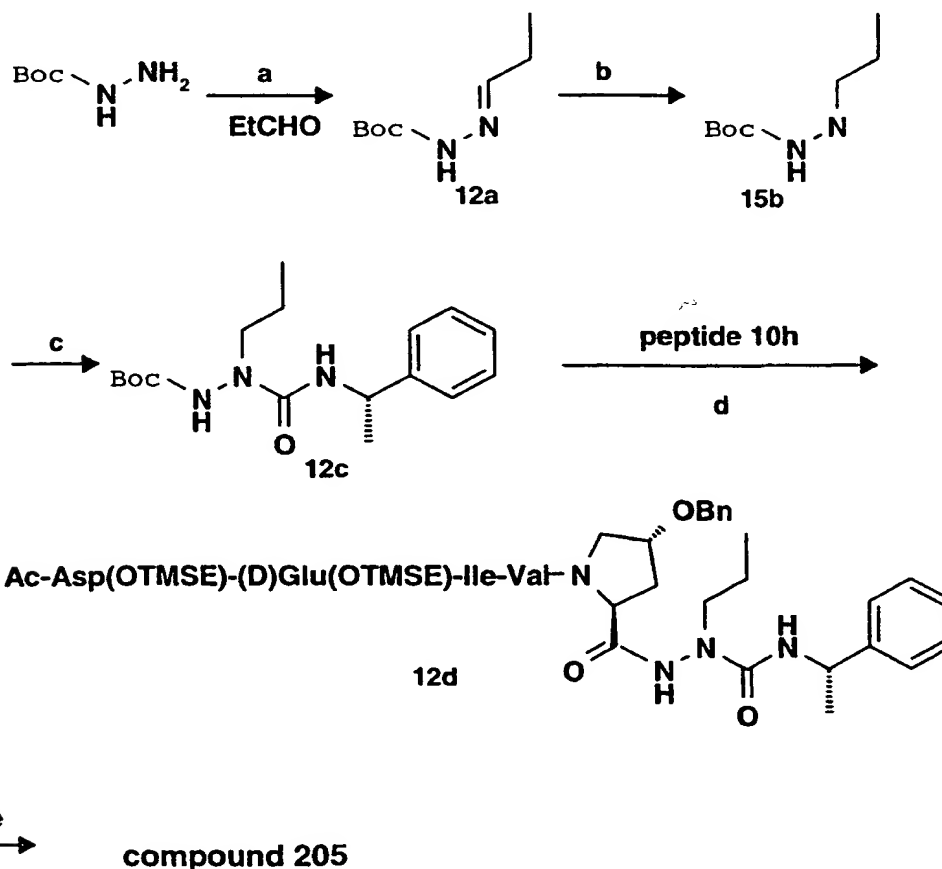3H), 2.02-1.82 (m, 3H), 1.82 (s, 3H), 1.78-1.28 (m, 6H), 1.07-0.95 (m, 1H), 0.92-0.80 (m, 10H), 0.77-0.68 (m, 8H).

5   **Example 12**

Synthesis of compound 205 (Table 2).

205

Compound 205 was prepared according to the following
10   scheme:

e → **compound 205**

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a) Synthesis of compound 12a:

To a solution of Boc-hydrazine (3.0 g, 22.6 mmol) in toluene (42 mL) was added propionaldehyde (1.8 mL, 24.9 mmol). The solution was heated to 50°C for 1 h
5 and then stirred at RT for 24 h. The mixture was concentrated to give **12a** as a white solid (3.70 g, 95%) which was homogeneous by analytical HPLC (97.5%). MS (FAB) 173.1 (MH$^+$); ^{1}H-NMR (DMSO-d$_6$) d 10.33 (bs, 1H), 7.28 (bs, 1H), 2.19-2.09 (m, 2H),
10 1.42 and 1.41 (2 x s, 9H), 0.97 (dt, J = 7.6, 1.6 Hz, 3H).

b) Synthesis of compound 12b:

To the hydrazone **12a** (3.7 g, 21.48 mmol) in THF (80
15 mL) at -78°C was added DIBAL (31 mL, 47.25 mmol) as a 1.5 M solution in toluene. The reaction was maintained at -78°C for 2 h and then -40°C for 2 h. Rochelle's salt (aqueous potassium sodium tartrate) solution was added and the reaction mixture stirred
20 at RT overnight. The organic phase was separated and the aqueous phase extracted with Et$_2$O (2 x 75 mL). The combined organic extracts were washed with brine, dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel
25 gave **12b** as a colourless oil (3.4 g, 91%). MS (CI-NH$_3$) 175.2 (MH$^+$); ^{1}H-NMR (DMSO-d$_6$) d 8.10 (bs, 1H), 4.25 (bs, 1H), 2.6 (t, J = 7.0 Hz, 2H), 1.45-1.29 (m, 2H), 1.38 (s, 9H), 0.85 (t, J = 7.6 Hz, 3H).

c) Synthesis of compound 12c:

30 The hydrazine **12b** (0.20 g, 1.15 mmol) was combined with (S)-(-)-1-phenylethylisocyanate (0.162 g, 1.15 mmol) in CH$_2$Cl$_2$ (2.4 mL) with DIPEA (0.44 mL, 2.52 mmol) and stirred at 0°C for 1 h, and then at RT for

3 h. The mixture was concentrated *in vacuo* to give a
white solid which was purified by flash
chromatography to give compound **12c** (0.25 g, 68%). MS
(FAB) 322.3 (MH$^+$); ^{1}H-NMR (DMSO-d$_6$) d 8.90 and 8.48 (2

5   x bs, 1H), 7.35-7.23 (m, 5H), 6.84 (t, J = 7.0 Hz,
1H), 6.50 (bs, 1H), 4.79 (t, J = 7.3 Hz, 1H), 1.41
(s, 9H), 1.36 (d, J = 7.0 Hz, 6H), 0.81 (t, J = 7.3
Hz, 3H).

10   **d) Synthesis of compound 12d:**
Compound **12c** (77 mg, 0.24 mmol) was treated with 4 N
HCl/dioxane (1.2 mL) for 20 min before being
concentrated *in vacuo*. The hydrochloride salt was
combined with the pentapeptide **10h** (0.20 g, 0.22

15   mmol), TBTU (85 mg, 0.26 mmol), and
diisopropylethylamine (0.13 mL, 0.73 mmol) in DMF
(2.5 mL) at RT for 16 h. The reaction mixture was
concentrated and the residue dissolved in EtOAc and
washed sequentially with saturated aqueous NaHCO$_3$,

20   10% aqueous HCl, and brine before being dried
(MgSO$_4$), filtered, and concentrated *in vacuo*.
Purification by flash chromatography gave **12d** as a
white solid (80 mg, 33%).

25   **e) Synthesis of compound 205:**
The protected peptide **12d** (75 mg, 0.067 mmol) was
treated with neat TFA (1.5 mL) for 1.5 h before being
concentrated *in vacuo*. Purification by preparative
HPLC gave **205** as a white solid (17 mg, 27%). HPLC

30   (98%); MS (FAB) 923.6 (MH$^+$); HRMS calcd for C$_{46}$H$_{66}$N$_8$O$_{12}$
(MH$^+$) 923.48785, found: 923.49097. ^{1}H-NMR (DMSO-d$_6$) d
12.5-11.9 (bs, 2H), 10.31 (bs, 1H), 8.26 (d, J = 7.95
Hz, 1H), 8.14 (d, J = 7.6 Hz, 1H), 7.98 (d, J = 8.26
Hz, 1H), 7.78 (d, J = 8.58 Hz, 1H), 7.42-7.26 (m,

10H), 7.20 (t, J = 7.0 Hz, 1H), 6.86 (bd, 1H), 4.85-
4.77 (m, 1H), 4.55 (d, J = 11.4 Hz, 1H), 4.50-4.40
(m, 1H), 4.46 (d, J = 11.8 Hz, 1H), 4.36-4.20 (m,
6H), 3.75-3.68 (m, 1H), 3.48-3.34 (bs, 1H), 3.18-3.07
5   (bs, 1H), 2.62 (dd, J = 16.5, 5.7 Hz, 1H), 2.44 (dd,
J = 16.5, 5.7 Hz, 1H), 2.30-2.22 (m, 1H), 2.17 (t, J
= 7.95 Hz, 2H), 2.06-1.84 (m, 2H), 1.81 (s, 3H),
1.80-1.59 (m, 2H), 1.42-1.30 (m, 6H), 1.06-0.98 (m,
1H), 0.88 (t, J = 7.0 Hz, 6H), 0.78 (t, J = 7.3 Hz,
10   3H), 0.71 (t, J = 7.0 Hz, 6H).

Example 13

Synthesis of compound 231

Compound **231** was prepared according to the procedure
15   for compound **205** except that the isocyanate was
replaced by the corresponding isocyanate prepared
from piperonylamine in the following manner.
To a solution of phosgene in toluene (0.2 mL, 0.38
mmol, 4 equiv.) and THF (0.7 mL) at 0°C was added
20   piperonylamine (12 µL, 0.095 mmol) in THF (0.7 mL)
containing diisopropylethylamine (53 µL, 0.30 mmol)
dropwise over a period of 15 min. The mixture was
stirred at 0°C for 30 min before being concentrated.
The generated isocyanate was coupled as described for
25   compound **12c**. The Boc group was removed (4 N
HCl/dioxane) and the hydrochloride salt of fragment
P1/P1' was coupled in the usual fashion with HATU,
diisopropylethylamine and the pentapeptide **10h** (0.10
g, 0.095 mmol) in DMF. The reaction mixture was
30   stirred at 0°C for 1 h and then at RT for 16 h. The
reaction mixture was concentrated and the residue
dissolved in EtOAc and washed sequentially with
saturated aqueous NaHCO$_3$, 10% aqueous HCl, and brine
before being dried (MgSO$_4$), filtered and concentrated

in vacuo. Purification by flash chromatography gave the desired protected peptide (67 mg, 62.5%).

The protected peptide (63 mg, 0.056 mmol) was treated
5   as for the synthesis of compound **12d** to give after
purification by preparative HPLC compound **231** as a
white solid (17 mg, 32%). HPLC (100%); MS (FAB)
953.05 (MH$^+$); HRMS calcd for $C_{46}H_{64}N_8O_{14}$ (MH$^+$)
953.46204, found: 953.46680. ^{1}H-NMR (DMSO-d$_6$) δ 10.35
10   (bs, 1H), 8.17 (d, J = 8.6 Hz, 1H), 8.15 (d, J = 7.95
Hz, 1H), 7.98 (d, J = 7.95 Hz, 1H), 7.76 (d, J = 8.6
Hz, 1H), 7.40-7.25 (m, 5H), 7.23-7.13 (bs, 1H), 6.82
(s, 1H), 6.79 (d, J = 7.6 Hz, 1H), 6.72 (d, J = 7.6
Hz, 1H), 5.95 (s, 2H), 4.57-4.43 (m, 3H), 4.34-4.18
15   (m, 6H), 4.13-4.08 (m, 2H), 3.70 (d, J = 3.8 Hz, 1H),
3.67 (d, J = 3.8 Hz, 1H), 2.68-2.58 (m, 2H), 2.48-
2.42 (m, 1H), 2.34-2.24 (m, 2H), 2.2-2.13 (m, 2H),
2.05-1.94 (m, 1H), 1.94-1.82 (m, 1H), 1.81 (s, 3H),
1.75-1.61 (m, 2H), 1.45-1.30 (m, 3H), 1.03-0.94 (m,
20   1H), 0.87-0.75 (m, 9H), 0.74-0.67 (m, 6H).

Example 14

Synthesis of compound 232

Compound **12b** was coupled to the appropriate
25   carboxylic acid (from Maybridge) using TBTU and
diisopropylethylamine in DMF in the usual fashion.
The Boc group from the P1/P1' fragment was removed (4
N HCl/dioxane, 30 min) and the corresponding
hydrochloride salt (0.104 mmol) coupled to peptide
30   **10h** (0.10 g, 0.095 mmol) in the usual fashion with
HATU (39.5 mg, 0.104 mmol) and diisopropylethylamine
(0.07 mL, 0.4 mmol) in DMF (0.95 mL) for 16 h. The
mixture was concentrated and the residue extracted
into EtOAc and washed with saturated aqueous NaHCO$_3$,

10% aqueous HCl and brine before being dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* to give the protected peptide (110 mg, 100%). Final deprotection of this peptide was accomplished by

5   saponification. The protected peptide (0.105 mg, 0.09 mmol) was dissolved in THF (1.3 mL), MeOH (0.7 mL) and H$_2$O (0.7 mL) before being treated with aqueous NaOH (0.18 mL of a 2 N solution, 0.36 mmol). The mixture was stirred for 3.5 h before being

10   concentrated *in vacuo*. The crude residue was purified by preparative HPLC to give compound **232** as a white solid (33 mg, 36%). HPLC (97%); MS (FAB) 977.4 (MH$^+$), 999.4 (MNa$^+$); HRMS calcd for C$_{47}$H$_{64}$N$_{10}$O$_{13}$ (MH$^+$) 977.47327, found: 97747620. ^{1}H-NMR (DMSO-d$_6$) δ 10.61

15   (bs, 1H), 8.75 (d, J = 4.7 Hz, 1H), 8.14 (d, J = 8.6 Hz, 1H), 8.06 (d, J = 7.6 Hz, 1H), 8.02-7.90 (m, 3H), 7.87 (d, J = 8.6 Hz, 1H), 7.60-7.56 (m, 1H), 7.35-7.26 (m, 5H), 4.57-4.43 (m, 4H), 4.42-4.34 (m, 2H), 4.33-4.25 (m, 2H), 4.25-4.17 (m, 2H), 3.70 (dd, J =

20   10.5, 10.5 Hz, 1H), 3.21-3.10 (m, 2H), 2.96-2.80 (m, 1H), 2.69-2.60 (m, 2H), 2.48-2.41 (m, 1H), 2.35-2.25 (m, 1H), 2.22-2.15 (m, 2H), 2.05-1.90 (m, 2H), 1.88-1.81 (m, 1H), 1.80 (s, 3H), 1.77-1.61 (m, 2H), 1.53-1.40 (m, 2H), 1.39-1.28 (m, 1H), 1.06-0.95 (m, 1H),

25   0.91-0.81 (m, 7H), 0.79 (t, J = 7.3 Hz, 3H), 0.75-0.67 (m, 6H).

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Example 15

Synthesis of compound 233
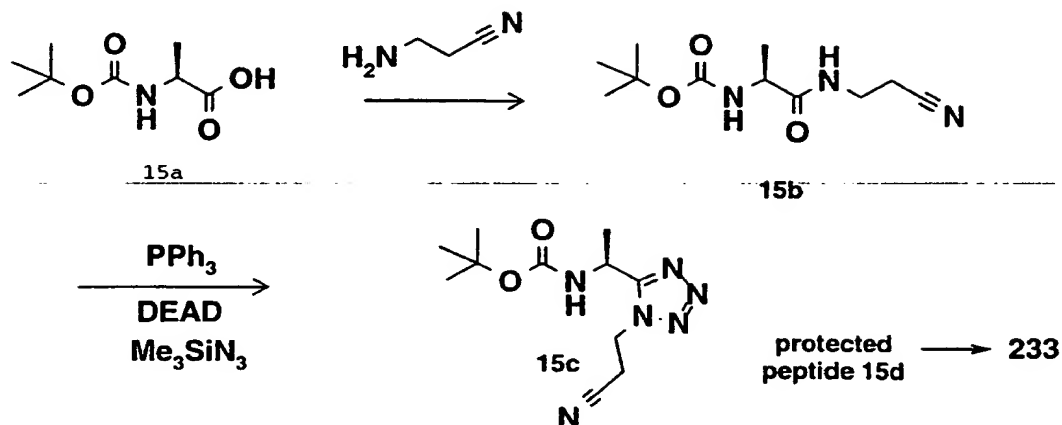
Synthesis of compound **15b**:

Boc-Alanine (**15a**) (5 g, 26.42 mmol) was dissolved in anhydrous DMF (63 mL) at 0°C before 3-aminopropionitrile (1.9 mL, 26.42 mmol) and HOBt (3.5 g, 26.42 mmol) were added. To this solution was added DCC (26.4 mL, 26.4 mmol, 1.0 M in dichloromethane) via syringe. The mixture was stirred at 0°C for 24 h. The generated DCU was filtered out through a pad of Celite and washed with cold dichloromethane. The filtrate was concentrated *in vacuo* and the residue re-dissolved in EtOAc and washed with 1N HCl (aqueous), saturated NaHCO$_3$, and saturated brine. The organic phase was dried (Na$_2$SO4), filtered and concentrated *in vacuo* to give 6.3 g of a pale brownish solid. Purification by flash chromatography using EtOAc/hexane (7/3) gave compound **15b** as a white solid (3.94 g, 62%). HPLC (95%); MS (FAB) 242.1 (MH$^+$), 264.1 (MNa$^+$); ^{1}H-NMR (DMSO-d$_6$) δ 8.10 (bs, 1H), 6.88 (d, J = 7 Hz, 1H), 3.86 (m, 1H), 3.35-3.2 (m, 2H), 1.37 (s, 9H), 1.18 (d, J = 7.3 Hz, 2H).

Synthesis of compound **15c**:

To a solution of compound **15b** (3.92 g, 17.5 mmol) in
THF (350 mL) at 0°C was added sequentially
triphenylphosphine (7.2 g, 35.4 mmol), DEAD (5.5 mL,
35.1 mmol), and trimethylsilylazide (4.66 mL, 35.1

5   mmol). The reaction was warmed to RT and left to stir
16 h. The mixture was then heated to 70°C for 4 h and
stirred an additional 48 h at RT. The solution was
cooled to 0°C and treated with excess 5.5% aqueous
$(NH_4)_2Ce(NO_3)_6$ [caution, add slowly dropwise]. The

10   crude mixture was extracted with EtOAc (3 x 100 mL)
and washed with saturated brine before being dried
(Na_2SO_4), filtered and concentrated *in vacuo*.
Purification by flash chromatography using
EtOAc/hexane (6/4) gave compound **15c** as a white solid

15   (3.3 g, 76%). MS (FAB) 267.1 (MH^+); ^1H-NMR (DMSO-d_6) δ
7.75 (d, J = , 1H), 5.1-5.02 (m, 1H), 4.73 (t, J =
2H), 3.18 (t, J = 2H), 1.49 (d, J = X, 3H), 1.36 (s,
9H).

20   Final deprotection of the tetrazole and the ester
functionalities was accomplished by saponification.
The protected peptide **15d** (38 mg, 0.033 mmol) was
dissolved in THF (0.5 mL), MeOH (0.25 mL) and H_2O
(0.25 mL) before being treated with aqueous NaOH (0.1

25   mL of a 2 N solution, 0.198 mmol) for 4 h. The
mixture was concentrated *in vacuo* and the crude
product purified by preparative HPLC to give after
lyophilization compound **233** as a white solid (7.5 mg,
25%). HPLC (98%); MS (FAB) 913.5 ($M-H^-$); HRMS calcd

30   for $C_{41}H_{62}N_{12}O_{12}$ (MH^+) 915.4688, found: 915.47250. ^1H-
NMR (DMSO-d_6) δ 10.32 (bs, 1H), 8.23 (d, J = 7.6 Hz,
1H), 8.05 (d, J = 7.6 Hz, 1H), 7.99 (d, J = 8.6 Hz,
1H), 7.90 (d, J = 9.2 Hz, 1H), 7.35-7.27 (m, 5H),
6.99 (m, 1H), 5.12 (m, 1H), 4.56-4.42 (m, 4H), 4.41-

4.34 (m, 1H), 4.34-4.17 (m, 5H), 3.70 (dd, J = 10.5, 10.5 Hz, 1H), 3.15 (bm, 1H), 2.67-2.61 (m, 2H), 2.48-2.40 (m, 1H), 2.30-2.23 (m, 1H), 2.22-2.15 (m, 2H), 2.02 -1.92 (m, 2H), 1.87-1.81 (m, 1H), 1.80 (s, 3H),

5   1.77-1.67 (m, 1H), 1.67-1.57 (m, 1H), 1.51 (d, J = 7 Hz, 3H), 1.45-1.27 (m, 3H), 1.06-0.94 (m, 1H), 0.87 (dd, J = 6.4, 6.4 Hz, 6H), 0.82 (t, J = 7.0 Hz, 3H), 0.72-0.62 (m, 6H).

10   **Example 16**

Synthesis of compound 107

To Boc-(L)Nvl-OH (0.28 g, 1.28 mmol) was added benzylamine (0.163 g, 1.53 mmol), TBTU (0.45 g, 1.41 mmol), and diisopropylethylamine (0.45 mL, 2.56 mmol)

15   in DMF (10 mL) for 16 h. The reaction was concentrated and the residue dissolved in EtOAc (80 mL) and washed sequentially with saturated aqueous NaHCO$_3$, 10% aqueous HCl, and brine before being dried (MgSO$_4$), filtered and concentrated *in vacuo* to give a

20   white solid (0.18 g, 46%) which was 91% homogeneous by analytical HPLC. This material (37 mg, 0.12 mmol) was treated with 4 N HCl/dioxane (5 mL) for 30 min before being concentrated *in vacuo*. The hydrochloride salt (0.12 mmol) was combined with the pentapeptide

25   **10h** (0.10 g, 0.11 mmol), TBTU (39 mg, 0.12 mmol) and diisopropylethylamine (0.07 mL, 0.38 mmol) in DMF (8 mL) and stirred for 16 h. The reaction mixture was concentrated and the residue dissolved in EtOAc and washed sequentially with saturated aqueous NaHCO$_3$,

30   10% aqueous HCl, and brine before being dried (MgSO$_4$), filtered, and concentrated *in vacuo* to give a white solid (0.12 g, 89%). The peptide was deprotected with neat TFA (5 mL) for 1 h before being

concentrated. The compound was purified by preparative HPLC to give compound **107** (39 mg, 39%). HPLC (98.5%); FAB MS m/z: 908 (MH$^+$); HRMS calcd for $C_{46}H_{65}N_7O_{12}$ (MH$^+$) 908.47693, found: 908.47230; AAA OK;

5   ^{1}H-NMR (DMSO-d$_6$) • 12.5-11.9 (bs, 2H), 8.30 (t, J = 5.7 Hz, 1H), 8.15 (d, J = 7.3 Hz, 1H), 8.02 (m, 3H), 7.79 (d, J = 6.8 Hz, 1H), 7.37-7.19 (m, 10H), 4.57-4.39 (m, 3H), 4.33-4.14 (m, 6H), 4.11 (d, J = 11.5 Hz, 1H), 3.68 (dd, J = 10.8, 4.1 Hz, 1H), 2.63 (dd, J

10   = 16.2, 6.2 Hz, 1H), 2.45 (dd, J = 16.2, 6.2 Hz, 1H), 2.23-2.15 (m, 3H), 2.02-1.85 (m, 3H), 1.82 (s, 3H), 1.79-1.67 (m, 2H), 1.66-1.48 (m, 2H), 1.40-1.23 (m, 3H), 1.08-0.97 (m, 1H), 0.92-0.81 (m, 11H), 0.73 (t, J = 7.95 Hz, 6H).

15

Example 17

Synthesis of compound 108

Compound **108** was prepared according to the procedure for compound **107**.

20   HPLC (99.9%); FAB MS m/z: 922 (MH$^+$); HRMS calcd for $C_{47}H_{67}N_7O_{12}$ (MH$^+$) 922.49261, found: 922.49560; ^{1}H-NMR (DMSO-d$_6$) • 12.5-11.9 (bs, 2H), 8.21 (d, J = 7.95 Hz, 1H), 8.15 (d, J = 7.6 Hz, 1H), 8.05 (d, J = 7.6 Hz, 2H), 7.91 (d, J = 7.95 Hz, 1H), 7.79 (8.9 Hz,

25   1H), 7.36-7.25 (m, 10H), 7.23-7.17 (m, 1H), 4.92-4.83 (m, 1H), 4.53 (m, 1H), 4.51 (d, J = 7.95 Hz, 1H), 4.44 (d, J = 7.95 Hz, 1H), 4.42 (m, 1H), 4.33-4.25 (m, 2H), 4.24-4.14 (m, 3H), 4.11 (d, J = 11 Hz, 1H), 3.71 (m, 1H), 2.63 (dd, J = 11.0, 4.0 Hz, 1H), 2.45

30   (dd, J = 11.0, 4.0 Hz, 1H), 2.23-2.14 (m, 3H), 2.04-1.86 (m, 3H), 1.82 (s, 3H), 1.80-1.66 (m, 2H), 1.62-1.42 (m, 2H), 1.33 (d, J = 7.0 Hz, 3H), 1.30-1.17 (m, 2H), 1.07-0.96 (m, 1H), 0.88 (m, 6H), 0.81 (t, J = 7.3 Hz, 3H), 0.73 (t, J = 7.6 Hz, 6H).

Example 18

RECOMBINANT HCV NS3 PROTEASE RADIOMETRIC ASSAY

5   a) <u>Cloning, expression and purification of the</u>
       <u>recombinant HCV NS3 protease type 1b</u>

       Serum from an HCV-infected patient was obtained
       through an external collaboration (Bernard Willems
10   MD, Hôpital St-Luc, Montréal, Canada and Dr. Donald
       Murphy, Laboratoire de Santé Publique du Québec, Ste-
       Anne de Bellevue, Canada). An engineered full-length
       cDNA template of the HCV genome was constructed from
       DNA fragments obtained by reverse transcription-PCR
15   (RT-PCR) of serum RNA and using specific primers
       selected on the basis of homology between other
       genotype 1b strains. From the determination of the
       entire genomic sequence, a genotype 1b was assigned
       to the HCV isolate according to the classification of
20   Simmonds et al. (J. Clin. Microbiol., (1993), <u>31</u>,
       p.1493-1503). The amino acid sequence of the non-
       structural region, NS2-NS4B, was shown to be greater
       than 93% identical to HCV genotype 1b (BK, JK and 483
       isolates) and 88% identical to HCV genotype 1a (HCV-1
25   isolate). A DNA fragment encoding the polyprotein
       precursor (NS3/NS4A/NS4B/NS5A/NS5B) was generated by
       PCR and introduced into eucaryotic expression
       vectors. After transient transfection, the
       polyprotein processing mediated by the HCV NS3
30   protease was demonstrated by the presence of the
       mature NS3 protein using Western blot analysis. The
       mature NS3 protein was not observed with expression
       of a polyprotein precursor containing the mutation

S1165A, which inactivates the NS3 protease,
confirming the functionality of the HCV NS3 protease.

The DNA fragment encoding the recombinant HCV NS3
5   protease (amino acid 1027 to 1206) was cloned in the
pET11d bacterial expression vector. The NS3 protease
expression in *E. coli* BL21(DE3)pLysS was induced by
incubation with 1 mM IPTG for 3 h at 22°C. A typical
fermentation (18 L) yielded approximately 100 g of
10 wet cell paste. The cells were resuspended in lysis
buffer (3.0 mL/g) consisting of 25 mM sodium
phosphate, pH 7.5, 10% glycerol (v/v), 1 mM EDTA,
0.01% NP-40 and stored at -80°C. Cells were thawed
and homogenized following the addition of 5 mM DTT.
15 Magnesium chloride and DNase were then added to the
homogenate at final concentrations of 20 mM and 20 μ
g/mL respectively. After a 25 min incubation at 4°C,
the homogenate was sonicated and centrifuged at 15000
x *g* for 30 min at 4°C. The pH of the supernatant was
20 then adjusted to 6.5 using a 1M sodium phosphate
solution.

An additional gel filtration chromatography step was
added to the 2 step purification procedure described
25 in WO 95/22985 (incorporated herein by reference).
Briefly, the supernatant from the bacterial extract
was loaded on a SP HiTrap column (Pharmacia)
previously equilibrated at a flow rate of 2 mL/min in
buffer A (50 mM sodium phosphate, pH 6.5, 10%
30 glycerol, 1 mM EDTA, 5 mM DTT, 0.01% NP-40). The
column was then washed with buffer A containing 0.15
M NaCl and the protease eluted by applying 10 column
volumes of a linear 0.15 to 0.3 M NaCl gradient. NS3
protease-containing fractions were pooled and diluted

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to a final NaCl concentration of 0.1 M.   The enzyme
was further purified on a HiTrap Heparin column
(Pharmacia) equilibrated in buffer B (25 mM sodium
phosphate, pH 7.5, 10% glycerol, 5 mM DTT, 0.01% NP-
5   40).   The sample was loaded at a flow rate of 3
mL/min.   The column was then washed with buffer B
containing 0.15 M NaCl at a flow rate of 1.5 mL/min.
Two step washes were performed in the presence of
buffer B containing 0.3 or 1M NaCl.   The protease was
10   recovered in the 0.3M NaCl wash, diluted 3-fold with
buffer B, reapplied on the HiTrap Heparin column and
eluted with buffer B containing 0.4 M NaCl.   Finally,
the NS3 protease-containing fractions were applied on
a Superdex 75 HiLoad 16/60 column (Pharmacia)
15   equilibrated in buffer B containing 0.3 M NaCl.   The
purity of the HCV NS3 protease obtained from the
pooled fractions was judged to be greater than 95% by
SDS-PAGE followed by densitometry analysis.

20   The enzyme was stored at -80°C and was thawed on ice
and diluted just prior to use.

Example 19

RECOMBINANT HCV NS3 PROTEASE/NS4A COFACTOR PEPTIDE
25   **RADIOMETRIC ASSAY**

The enzyme was cloned, expressed and prepared
according to the protocol described in Example 18.
The enzyme was stored at -80°C, thawed on ice and
30   diluted just prior to use in the assay buffer
containing the NS4A cofactor peptide.

The substrate used for the NS3 protease/N24A cofactor
peptide radiometric assay, DDIVPC-SMSYTW, is cleaved

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between the cysteine and the serine residues by the enzyme. The sequence DDIVPC-SMSYTW corresponds to the NS5A/NS5B natural cleavage site in which the cysteine residue in P2 has been substituted for a proline. The

5   peptide substrate DDIVPC-SMSYTW and the tracer biotin-DDIVPC-SMS[^{125}I-Y]TW are incubated with the recombinant NS3 protease and the NS4A peptide cofactor KKGSVVIVGRIILSGRK (molar ratio enzyme: cofactor 1:100) in the absence or presence of

10 inhibitors. The separation of substrate from products is performed by adding avidin-coated agarose beads to the assay mixture followed by filtration. The amount of SMS[^{125}I-Y]TW product found in the filtrate allows for the calculation of the percentage of substrate

15 conversion and of the percentage of inhibition.

A. Reagents

Tris and Tris-HCl (UltraPure) were obtained from

20 Gibco-BRL. Glycerol (UltraPure), MES and BSA were purchased from Sigma. TCEP was obtained from Pierce, DMSO from Aldrich and NaOH from Anachemia.

Assay buffer: 50 mM Tris HCl, pH 7.5, 30% (w/v)

25 glycerol, 1 mg/mL BSA, 1 mM TCEP (TCEP added just prior to use from a 1 M stock solution in water).

Substrate: DDIVPCSMSYTW, 25 μM final concentration (from a 2 mM stock solution in DMSO stored at -20°C

30 to avoid oxidation).

Tracer: reduced mono iodinated substrate biotin DDIVPC SMS[^{125}I Y]TW (~1 nM final concentration).

HCV NS3 protease type 1b, 25 nM final concentration
(from a stock solution in 50 mM sodium phosphate, pH
7.5, 10% glycerol, 300 mM NaCl, 5 mM DTT, 0.01% NP-
40).

5

NS4A Cofactor peptide: KKGSVVIVGRIILSGRK, 2.5 μM
final concentration (from a 2 mM stock solution in
DMSO stored at -20°C).

10 B. Protocol

The assay was performed in a 96-well polypropylene
plate from Costar. Each well contained:

- 20 μL substrate/tracer in assay buffer;
15
- 10 μL ± inhibitor in 20% DMSO/assay buffer;

- 10 μL NS3 protease 1b/NS4 cofactor peptide (molar
  ratio 1:100).

Blank (no inhibitor and no enzyme) and control (no
20 inhibitor) were also prepared on the same assay
plate.

The enzymatic reaction was initiated by the addition
of the enzyme/NS4A peptide solution and the assay
25 mixture was incubated for 40 min at 23°C under gentle
agitation. Ten (10) μL of 0.5N NaOH were added and
10 μL 1 M MES, pH 5.8 were added to quench the
enzymatic reaction.

30 Twenty (20) μL of avidin-coated agarose beads
(purchased from Pierce) were added in a Millipore
MADP N65 filtration plate. The quenched assay mixture

was transferred to the filtration plate, and
incubated for 60 min at 23°C under gentle agitation.

The plates were filtered using a Millipore
5   MultiScreen Vacuum Manifold Filtration apparatus, and
40 μL of the filtrate was transferred in an opaque
96-well plate containing 60 μL of scintillation fluid
per well.

10   The filtrates were counted on a Packard TopCount
instrument using a ^{125}I-liquid protocol for 1 minute.

The %inhibition was calculated with the following
equation:
15

$$100-[(counts_{inh}-counts_{blank})/(counts_{ctl}-counts_{blank}) \times 100]$$

A non-linear curve fit with the Hill model was
applied to the inhibition-concentration data, and the
20   50% effective concentration (IC_{50}) was calculated by
the use of SAS software (Statistical Software System;
SAS Institute, Inc., Cary, N.C.).

Example 20

25   **SPECIFICITY ASSAYS**

The specificity of the compounds was determined
against a variety of serine proteases: human
leukocyte elastase, porcine pancreatic elastase and
30   bovine pancreatic α-chymotrypsin and one cysteine
protease: human liver cathepsin B. In all cases a
96-well plate format protocol using a colorimetric p-
nitroanilide (pNA) substrate specific for each enzyme
was used. Each assay included a 1 h enzyme-inhibitor

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pre-incubation at 30°C followed by addition of substrate and hydrolysis to ≈30% conversion as measured on a UV Thermomax® microplate reader. Substrate concentrations were kept as low as possible compared to K_M to reduce substrate competition. Compound concentrations varied from 300 to 0.06 μM depending on their potency. The final conditions for each assay were as follows:

50mM Tris-HCl pH 8, 0.5 M Na_2SO_4, 50 mM NaCl, 0.1 mM EDTA, 3% DMSO, 0.01% Tween-20 with;

[100 μM Succ-AAPF-pNA and 250 pM α-chymotrypsin],
[133 μM Succ-AAA-pNA and 8 nM porcine elastase], [133 μM Succ-AAV-pNA and 8 nM leukocyte elastase]; or
[100 mM $NaHPO_4$ pH 6, 0.1 mM EDTA, 3% DMSO, 1mM TCEP, 0.01% Tween-20, 30 μM Z-FR-pNA and 5 nM cathepsin B (the stock enzyme was activated in buffer containing 20 mM TCEP before use)].

A representative example is summarized below for porcine pancreatic elastase:

In a polystyrene flat-bottom 96-well plate were added using a Biomek liquid handler (Beckman):

- 40 μL of assay buffer (50 mM Tris-HCl pH 8, 50 mM NaCl, 0.1 mM EDTA);

- 20 μL of enzyme solution (50 mM Tris-HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 40 nM porcine pancreatic elastase); and

- 20 μL of inhibitor solution (50 mM Tris-HCl, pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 1.5 mM-0.3 μM inhibitor, 15% v/v DMSO).

After 60 min pre-incubation at 30°C, 20 μL of substrate solution (50 mM Tris-HCl, pH 8, 0.5 M Na_2SO_4, 50 mM NaCl, 0.1 mM EDTA, 665 μM Succ-AAA-pNA) were added to each well and the reaction was further

5   incubated at 30°C for 60 min after which time the absorbance was read on the UV Thermomax® plate reader. Rows of wells were allocated for controls (no inhibitor) and for blanks (no inhibitor and no enzyme).

10

The sequential 2-fold dilutions of the inhibitor solution were performed on a separate plate by the liquid handler using 50 mM Tris-HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 15% DMSO. All other

15   specificity assays were performed in a similar fashion.

The percentage of inhibition was calculated using the formula:

20              $[1-((UV_{inh}-UV_{blank})/(UV_{ctl}-UV_{blank}))] \times 100$

A non-linear curve fit with the Hill model was applied to the inhibition-concentration data, and the 50% effective concentration (IC_{50}) was calculated by

25   the use of SAS software (Statistical Software System; SAS Institute, Inc., Cary, N.C.).

Example 21

Tables of compounds

30

The following tables list IC_{50} values of compounds representative of the invention.

The following abbreviations are used:

IC_{50}: The concentration required to obtain 50% inhibition in the NS3 protease/NS4A cofactor peptide radiometric assay according to Example 19.

5   **HLE**: The concentration required to obtain 50% inhibition in the human leukocyte elastase assay;

PPE: The concentration required to obtain 50% inhibition in the porcine pancreatic elastase assay;

Other: Figures unmarked indicate the concentration

10   required to obtain 50% inhibition in the bovine pancreatic α-chymotrypsin assay; figures marked with ** indicate the concentration required to obtain 50% inhibition in the human liver cathepsin B assay; **MS**: Mass spectrometric data (MH^+ from FAB); **AAA**: amino

15   acid analysis data expressed in % peptide recovery; **Acpr**: 1-amino-cyclopropylcarboxylic acid; **Acpe**: 1-amino-cyclopentylcarboxylic acid; **Abu**: aminobutyric acid; **Chg**: cyclohexylglycine (2-amino-2-cyclohexyl-acetic acid); **Hyp**: 4*(R)*-hydroxyproline; **Hyp(4-Bn)**:

20   4*(R)*-benzyloxyproline; **Pip**: pipecolic acid; **Tbg**: *tert*-butylglycine; **Ac**: acetyl; **Bn**: benzyl; **O-Bn**: benzyloxy; **DAD**: 3-carboxypropionyl; and **DAE**: 4-carboxybutyryl.

25

Table 1
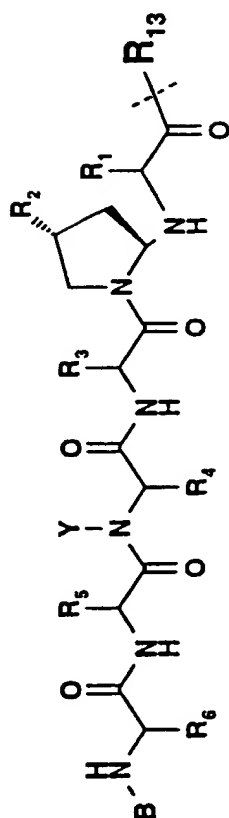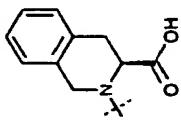
cpd	B	P6	P5	P4	P3	R_2	R_1	R_{13}	IC_{50} (μM)	HLE (μM)	PPE (μM)	OTHER (μM)	MS (MH⁺)	AAA (%)
101	Ac	Asp	D-Glu	Ile	Val	O-Bn	ethyl	CF_3	9.4	<1.2			857.4	
102	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	CF_2CF_3	0.21				921	99.2
103	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	C(O)NH-Bn	0.023				936	
104	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	H	0.14	7	8	8	803	115
105	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	NH_2	54				818	108
106	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	CH_2CH_2-Ph	5.4	16			908	107.8
107	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	$NHCH_2Ph$	3	3			908	100.8
108	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	(S)-NH-CH(Me)Ph	0.71	2	>300	>300	922	107
109	Ac	---	---	Chg	Val	O-Bn	propyl	C(O)-NHBn	23				718.3	
110	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	(structure)	8				(MH⁺) 753.5	

Table 1
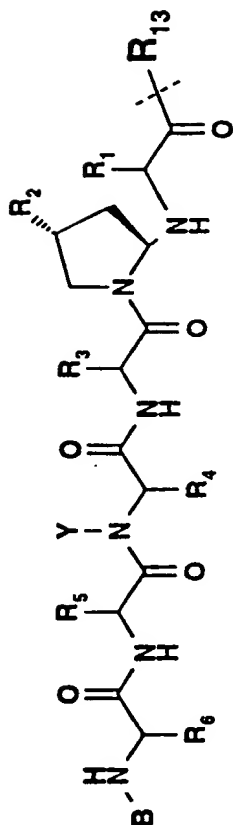
cpd	B	P6	P5	P4	P3	R_2	R_1	R_{13}	IC_{50} (µM)	HLE (µM)	PPE (µM)	OTHER (µM)	MS (MH⁺)	AAA (%)
111	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	(tetrahydroisoquinoline)	20					
112	Ac	Asp	D-Glu	Ile	Val	H	propyl	C(O)-NH-Bn	2	0.03	<0.06	10.3	814.4	104.4
113	Ac	Asp	D-Glu	Ile	Val	H	propyl	CF_2-CF_3	12.8	0.07	<0.06	18		
114	Ac	Asp	D-Glu	Ile	Val	H	propyl	CF_3	23.5	0.05	0.2	4.4	749.3	106.7
115	Ac	Asp	D-Glu	Ile	Val	H	propyl	C(O)NH Bn	0.66	0.11	<0.06	30	814	99.4
116	Ac	---	---	Chg	Val	O-Bn	propyl	C(O)NH-CH_2-4-pyridine	25					

Table 2
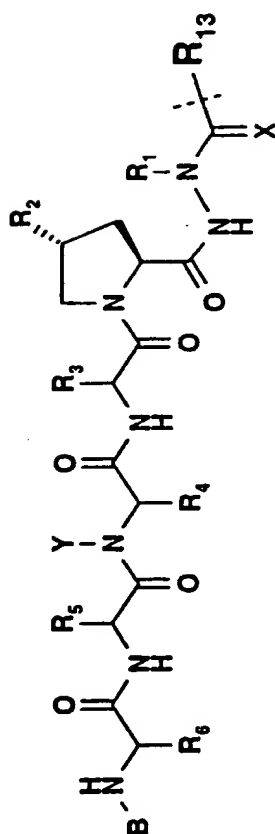
entry #	B	P6	P5	P4	P3	R2	R1	X	R13	IC50 (µM)	HLE (µM)	PPE (µM)	MS (MH+)	AAA (%)
201	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	NHCH2-C(O)OEt	3.9	27.4	168	905.2	
202	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	CH2CH2-Ph	0.63	5.5		908.4	
203	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	NHCH2-Ph	0.59			909.6	
204	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	(R)-NH-CH(Me)Ph	4.2			923.6	
205	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	(S)-NH-CH(Me)Ph	0.078	5.1	4.1	923.6	93.4
206	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	OCH2Ph	0.79	<0.6	<0.6	910	95.6
207	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	NHCH2-C(O)OH	3.75			877.1	106.5
208	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	CH3	14.5			818.2	101.9
209	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	NH2	20.5			819.3	100.6
210	Ac	Asp	D-Glu	Ile	Val	O-Bn	Butyl	O	(S)-NH-CH(Me)Ph	0.085	4		937.5	95.4
211	Ac	Asp	D-Glu	Ile	Val	O-Bn	Butyl	O	NH2	47			833	84

Table 2
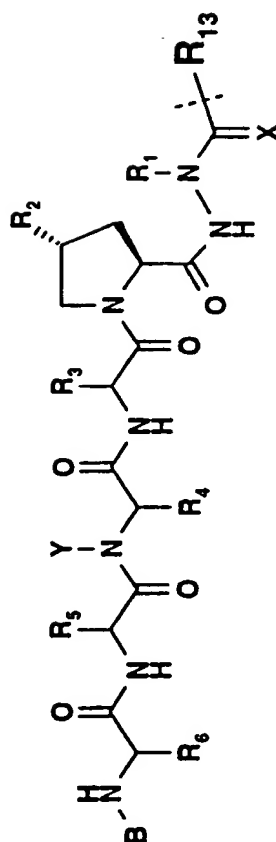
entry #	B	P6	P5	P4	P3	R_2	R_1	X	R_{13}	IC_{50} (µM)	HLE (µM)	PPE (µM)	MS (MH^+)	AAA (%)
212	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	(S)-NH-CH(Me)-naphtyl	0.58	0.4		974.3	98.9
213	Ac	Asp	D-Glu	Ile	Val	O-Bn	Ethyl	O	(S)-NH-CH(Me)Ph	0.079	36		909.9	103.3
214	Ac	Asp	D-Glu	Ile	Val	O-Bn	Propyl	O	(S)-NH-CH(Et)Ph	0.44			937.5	
215	Ac	---	---	Chg	Val	O-Bn	Propyl	O	(S)-NH-CH(Me)Ph	45	5		705.5	95.8
216	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O	(R)-CH(OH)Ph	9.6			(MH^+) 908.5	
217	Ac	Asp	D-Glu	Ile	Val	H	propyl	O	(S)-NH-CH(Me)Ph	1.04			803.4	93.2
218	Ac	Asp	D-Glu	Ile	Val	O-Bn	isopentyl	O	(S)-NH-CH(Me)Ph	3.3			951.5	99.5
219	Ac	Asp	D-Glu	ile	Val	O-Bn	pentyl	O	(S)-NH-CH(Me)Ph	0.43	151		951.5	
220	HOOC (CH2)2-C(O)	---	D-Asp	Ile	Val	H	propyl	O	(S)-NH-CH(Me)Ph	6.9			(MH^+) 744.5	97.5
221	Ac	Asp	D-Glu	Ile	Val	O-Bn	Me	O	(S)-NH-CH(Me)Ph	0.52	168		895	94
222	Ac	Asp	D-Glu	Ile	Val	O-Bn	(CH2)2-isopropyl	O	(S)-NH-CH(Me)Ph	8.9	>300		951.4	98.2
223	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O	N(CH$_3$)-Bn	11			923.5	105.5
224	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	S	NH-Bn	5			(MH^+)	

Table 2
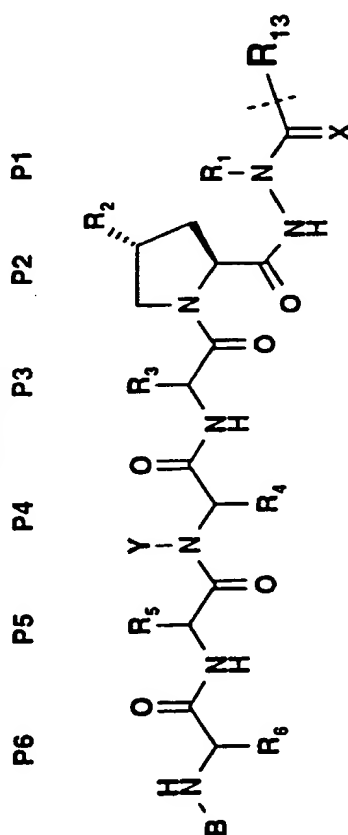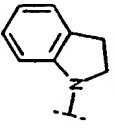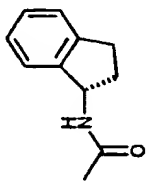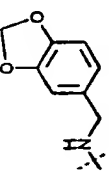
entry #	B	P6	P5	P4	P3	R2	R1	X	R13	IC50 (µM)	HLE (µM)	PPE (µM)	MS (MH+)	AAA (%)
225	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O	COCH3	13			923.4	102.3
226	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O	(indane)	6.3			862.3	112.8
227	Ac	---	---	Chg	Val	O-Bn	propyl	O	COOH	39.5			(MH+) 921.3 628.3	
228	Ac	Asp	D-Glu	Ile	Val	O-Bn	CH2-CF3	O	(S)-NH-CH(Me)Ph	0.63			(MH+) 961.4	
229	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O	(N-acetyl indane)	0.13	3.1		(MH+) 933.4	94.1
230	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O	(S)-NH-CH(Me) (benzodioxole)	0.36			929.5	100.6
231	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O		0.5			953.4	107.3

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Table 2
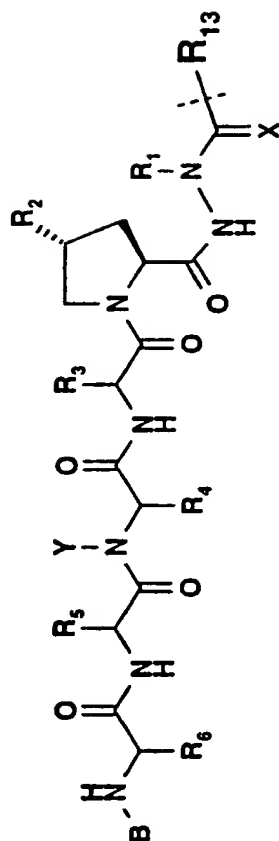
entry #	B	P6	P5	P4	P3	R2	R1	X	R13	IC50 (μM)	HLE (μM)	PPE (μM)	MS (MH⁺)	AAA (%)
232	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O		3.2			977.4	96.9
233	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O		1.5			(MH⁺) 913.5	
234	Ac	Asp	D-Glu	Ile	Val	O-Bn	CH₂-CH=CH₂	O	N-benzyl	3.7			907.5	

BNSDOCID: <WO___9907734A2_I_>

Table 3
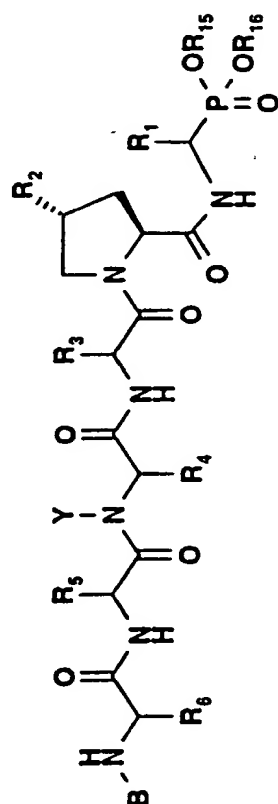
entry #	B	P6	P5	P4	P3	R2	R1	R15	R16	IC$_{50}$ (μM)	HLE (μM)	PPE (μM)	OTHER (μM)	MS (MH$^+$)	AAA (%)
301	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O-Ph	O-Ph	0.029	<0.6	<0.6	3 >300	1007	86.6
302	Ac	Asp	D-Glu	Ile	Val	O-Bn	propyl	O-Ph	O-Ph	26				1007	

What is claimed is:

1.   A compound of formula I:

P6   P5   P4   P3   P2   P1

(I)
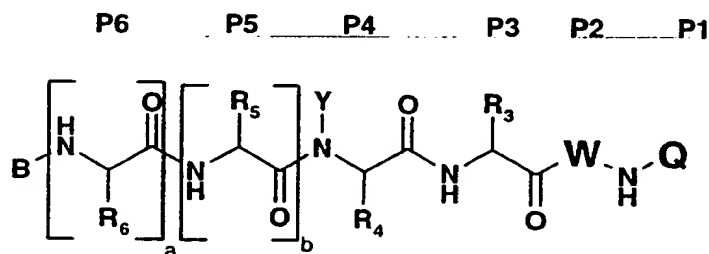
wherein **B** is an acyl derivative of formula R_{11}-C(O)-
wherein R_{11} is C_{1-10} alkyl optionally substituted with
carboxyl; or R_{11} is C_6 or C_{10} aryl or C_{7-16} aralkyl
optionally substituted with a C_{1-6} alkyl;

a is 0 or 1;

R_6, when present, is carboxy(lower)alkyl;

b is 0 or 1;

R_5, when present, is C_{1-6} alkyl, or
carboxy(lower)alkyl;

Y is H or C_{1-6} alkyl;

R_4 is C_{1-10} alkyl; C_{3-10} cycloalkyl;

R_3 is C_{1-10} alkyl; C_{3-10} cycloalkyl;

W is a group of formula II:

Formula II

wherein R_2 is C_{1-10} alkyl or C_{3-7} cycloalkyl optionally
substituted with carboxyl; C_6 or C_{10} aryl; or C_{7-16}
aralkyl; or

W is a group of formula II':

Formula II'

wherein **X** is CH or N; and

R$_2$' is C$_{3-4}$ alkylene that joins **X** to form a 5- or 6-membered ring, said ring optionally substituted with OH; SH; NH$_2$; carboxyl; **R$_{12}$**; OR$_{12}$, SR$_{12}$, NHR$_{12}$ or NR$_{12}$R$_{12}$'

wherein **R$_{12}$** and **R$_{12}$'** are independently:

cyclic C$_{3-16}$ alkyl or acyclic C$_{1-16}$ alkyl or cyclic C$_{3-16}$ alkenyl or acyclic C$_{2-16}$ alkenyl, said alkyl or alkenyl optionally substituted with NH$_2$, OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N; or

R$_{12}$ and **R$_{12}$'** are independently C$_6$ or C$_{10}$ aryl or C$_{7-16}$ aralkyl optionally substituted with C$_{1-6}$ alkyl, NH$_2$, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

said cyclic alkyl, cyclic alkenyl, aryl or aralkyl being optionally fused with a second 5-, 6-, or 7-membered ring to form a cyclic system or heterocycle, said second ring being optionally substituted with NH$_2$, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said second ring optionally containing at least one

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heteroatom selected independently from the group
consisting of: O, S, and N;

Q is a group of the formula:

$$
\begin{array}{c}
\mathbf{R_1} \\
| \\
\diagdown \mathbf{Z} \diagdown\!\!\!\diagup \mathbf{R_{13}} \\
\| \\
\mathbf{X}
\end{array}
$$

5   wherein **Z** is CH or N;

X is O or S;

R₁ is H, C_{1-6} alkyl or C_{1-6} alkenyl both optionally
substituted with thio or halo;
and

10   when **Z** is CH, then **R₁₃** is H; CF_3; CF_2CF_3; CH_2-**R₁₄**;
$CH(F)$-**R₁₄**; CF_2-**R₁₄**; $NR_{14}R_{14}'$ S-**R₁₄**; or CO-NH-**R₁₄** wherein
R₁₄ and **R₁₄'** are independently hydrogen, cyclic
C_{3-10} alkyl or acyclic C_{1-10} alkyl or cyclic C_{3-10}
alkenyl or acyclic C_{2-10} alkenyl, said alkyl or
15   alkenyl optionally substituted with NH_2, OH, SH,
halo or carboxyl; said alkyl or alkenyl
optionally containing at least one heteroatom
selected independently from the group consisting
of: O, S, and N; or
20   **R₁₄** and **R₁₄'** are independently C_6 or C_{10} aryl or
C_{7-16} aralkyl optionally substituted with C_{1-6}
alkyl, NH_2, OH, SH, halo, carboxyl or
carboxy(lower)alkyl or substituted with a
further C_{3-7} cycloalkyl, C_6 or C_{10} aryl, or
25   heterocycle; said aryl or aralkyl optionally
containing at least one heteroatom selected
independently from the group consisting of: O,
S, and N;
said cyclic alkyl, cyclic alkenyl, aryl or
30   aralkyl being optionally fused with a second 5-,
6-, or 7-membered ring to form a cyclic system

or heterocycle, said second ring being optionally substituted with NH_2, OH, SH, halo, carboxyl or carboxy(lower)alkyl or substituted with a further C_{3-7} cycloalkyl, C_6 or C_{10} aryl, or

5   heterocycle; said second ring optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

    or R_{14} and $R_{14'}$ are independently C_{1-4} alkyl which

10   when joined together with N form a 3 to 6-membered nitrogen-containing ring which is optionally fused with a further C_{3-7} cycloalkyl, C_6 or C_{10} aryl or heterocycle;

with the proviso that when Z is CH, then R_{13} is not

15   an α-amino acid or an ester thereof;

    when Z is N, then R_{13} is H; carboxy; C_{1-6} alkyl optionally substituted with carboxy; CH_2-R_{14}; $CHR_{14}R_{14'}$; CH(F)-R_{14}; O-R_{14}; $NR_{14}R_{14'}$ or S-R_{14} wherein R_{14}

20   and $R_{14'}$ are as defined above; or

    Q is a phosphonate group of the formula:
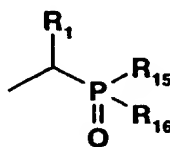
wherein R_{15} and R_{16} are independently C_{6-20} aryloxy; and

25   R_1 is as defined above.

    2.   A compound of formula I according to claim 1, wherein

    Q is a group of the formula:

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$$\underset{O}{\overset{R_1}{\underset{\|}{\overset{\|}{Z}}}}\quad R_{13}$$
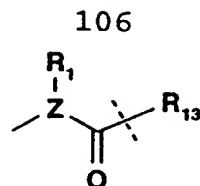
wherein Z is CH or N;

R_1 is hydrogen, C_{1-6} alkyl optionally substituted with thiol, or C_{1-6} alkenyl;

5   and

when Z is CH, then R_{13} is H; CF_3; CF_2CF_3; CH_2-R_{14}; $CH(F)-R_{14}$; CF_2-R_{14}; $NR_{14}R_{14}'$ $S-R_{14}$; $CHR_{14}R_{14}'$ or $CO-NH-R_{14}$ wherein

10   R_{14} and R_{14}' are independently hydrogen, cyclic C_{3-10} alkyl or acyclic C_{1-10} alkyl or cyclic C_{3-10} alkenyl or acyclic C_{2-10} alkenyl, said alkyl or alkenyl optionally substituted with NH_2, OH, SH, halo or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom
15   selected independently from the group consisting of: O, S, and N; or

R_{14} and R_{14}' are independently C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} alkyl, NH_2, OH, SH, halo, carboxyl or
20   carboxy(lower)alkyl; said aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

said cyclic alkyl, cyclic alkenyl, aryl or
25   aralkyl being optionally fused with a second 5-, 6-, or 7-membered ring to form a cyclic system or heterocycle, said second ring being optionally substituted with NH_2, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said second
30   ring optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

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with the proviso that, when **Z** is CH, then R_{13} is not

an α-amino acid or an ester thereof;

when **Z** is N, then R_{13} is H; CH_3; NH_2; CH_2-R_{14}; $CH(F)-$

R_{14}; $CHR_{14}R_{14}'$; $O-R_{14}$; $NH-R_{14}$; $NR_{14}R_{14}'$ or $S-R_{14}$ wherein

5   R_{14} and R_{14}' are as defined above.

---

3.   The compound of formula I according to claim 2,

wherein when **X** is N then R_2' is substituted with OH;

SH; NH_2; carboxyl; R_{12}; OR_{12}, SR_{12}, NHR_{12} or $NR_{12}R_{12}'$

10   wherein R_{12} and R_{12}' are as defined in claim 1.

4.   The compound of formula I according to claim 1,

wherein **B** is preferably an acyl derivative of formula

$R_{11}C(O)-$ wherein R_{11} is:

15   C_{1-6} alkyl optionally substituted with carboxyl, C_{1-6}

alkanoyloxy or C_{1-6} alkoxy;

C_{3-7} cycloalkyl optionally substituted with carboxyl,

MeOC(O), EtOC(O) or BnOC(O);

3-carboxypropionyl (DAD) or 4-carboxybutyryl (DAE);

20   or

$$\text{HOOCCH}_2\text{N} \quad \text{NCOOBn}$$
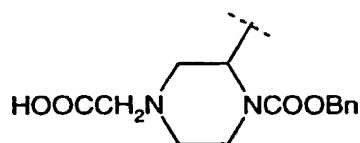
5.   The compound of formula I according to claim 4,

wherein **B** is acetyl, 3-carboxypropionyl, 4-

25   carboxylbutyryl, $AcOCH_2C(O)$, $Me_3COC(O)$,

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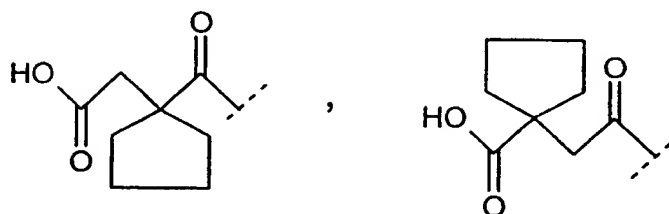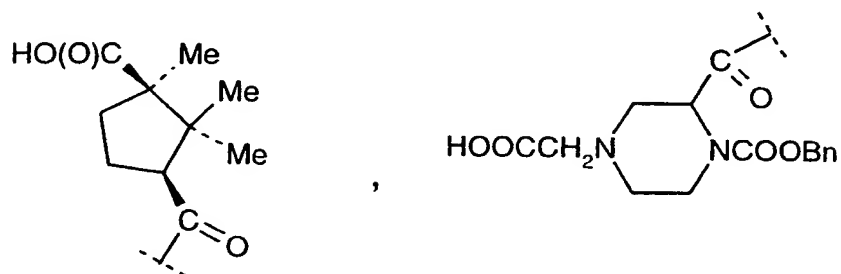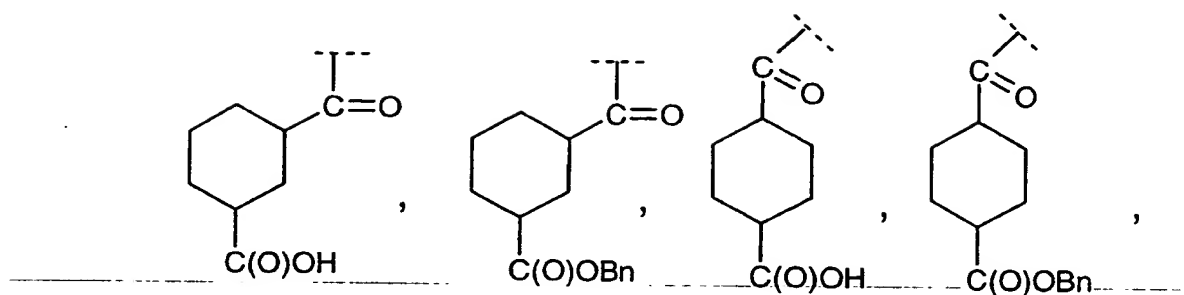
6.   The compound of formula I according to claim 5, wherein **B** is acetyl, 3-carboxypropionyl (DAD), 4-carboxybutyryl (DAE), AcOCH$_2$C(O),

or
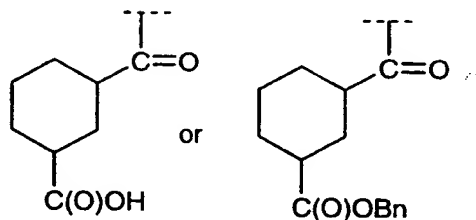
7.   The compound of formula I according to claim 6, wherein **B** is acetyl, or 4-carboxybutyryl (DAE).

8.   The compound of formula I according to claim 7, wherein **B** is acetyl.

9.   The compound of formula I according to claim 1 wherein R_6, when present, is the side chain of Asp or Glu.

10.   The compound of formula I according to claim 9, wherein R_6, when present, is the side chain of Asp.

11.   The compound of formula I according to claim 1, wherein **a** is 0 and then R_6 is absent.

12.   The compound of formula I according to claim 1, wherein R_5, when present, is the side chain of an amino acid selected from the group consisting of: D-Asp, L-Asp, D-Glu, L-Glu, D-Val, L-Val, D-*tert*-butylglycine (Tbg), and L-Tbg.

13.   The compound of formula I according to claim 12, wherein R_5, when present, is the side chain of D-Asp, D-Val, or D-Glu.

14.   The compound of formula I according to claim 13, wherein R_5, when present, is the side chain of D-Glu.

15.   The compound of formula I according to claim 1, wherein **a** is 0 and **b** is 0, and then both R_6 and R_5 are absent.

16.   The compound of formula I according to claim 1, wherein R_4 is the side chain of an amino acid selected from the group consisting of: Val, cyclohexylglycine (Chg), Tbg, Ile, or Leu.

17.   The compound of formula I according to claim 16, wherein R_4 is the side chain of Chg or Ile.

18.   The compound of formula I according to claim 17, wherein **R₄** is the side chain of Chg.

5   19.   The compound of formula I according to claim 1, wherein **R₃** is the side chain of an amino acid selected from the group consisting of: Ile, Chg, Cha, Val or Glu.

10   20.   The compound of formula I according to claim 19, wherein **R₃** is the side chain of Val or Chg.

21.   The compound of formula I according to claim 20, wherein **R₃** is the side chain of Val.

15

22.   The compound of formula I according to claim 1, wherein **W** is a group of formula II:
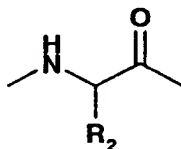
wherein **R₂** is C₁₋₈ alkyl or C₃₋₆ cycloalkyl optionally
20   substituted with carboxyl; or benzyl.

23.   The compound of formula I according to claim 22, wherein **R₂** is the side chain of Asp, aminobutyric acid (Abu) or Val.

25

24.   The compound of formula I according to claim 1, wherein **W** is a group of formula II':
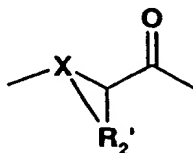
wherein preferably, **X** is CH or N; and

R₂' is a C_3 or C_4 alkylene that joins **X** to form a 5- or 6-membered ring of formula III:
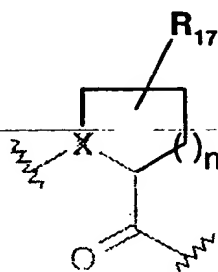
Formula III

R₂' being optionally substituted at any position with **R₁₇**;

wherein **X** is CH or N; **n** is 1 or 2; and

R₁₇ is OH; SH; NH_2; carboxyl; **R₁₂**; OR_{12}, SR_{12}, NHR_{12} or $NR_{12}R_{12}'$ wherein **R₁₂** and **R₁₂'** are independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl, said alkyl or alkenyl optionally substituted with NH_2, OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom independently selected from the group consisting of: O, S, and N; or

R₁₂ and **R₁₂'** are independently C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} alkyl, NH_2, OH, SH, halo, carboxyl or carboxy(lower)alkyl; said aryl or aralkyl optionally containing at least one heteroatom independently selected from the group consisting of: O, S, and N;

said cyclic alkyl, cyclic alkenyl, aryl or aralkyl being optionally fused with a second 5-, 6-, or 7-membered ring to form a cyclic system or heterocycle, said second ring being optionally substituted with NH_2, OH, SH, halo,

carboxyl or carboxy(lower)alkyl; said second ring optionally containing at least one heteroatom independently selected from the group consisting of: O, S, and N.

5

25. The compound of formula I according to claim 24, wherein X is N; and R_{17} is at the 3-, 4-, or 5-position of the ring.

10   26. The compound of formula I according to claim 25, wherein R_2' is the side chain of proline and is substituted with R_{17} at the 4-position with the stereochemistry shown in formula III':
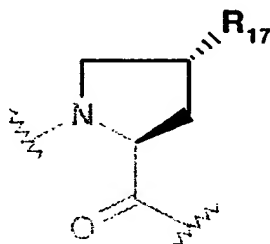
Formula III'

15   wherein R_{17} is OR_{12} wherein R_{12} is a C_6 or C_{10} aryl or C_{7-16} aralkyl, said first aryl or aralkyl optionally substituted with C_{1-6} alkyl, C_{3-7} cycloalkyl, NH_2, OH, SH, halo, C_{1-6} alkoxy, carboxyl, carboxy(lower)alkyl, or a second aryl or aralkyl; said first and second 20   aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N.

25   27. The compound of formula I according to claim 26, wherein R_{17} is Bn, $PhCH_2CH_2$, $PhCH_2CH_2CH_2$, O-Bn, o-tolylmethoxy, m-tolylmethoxy, p-tolylmethoxy, 1-naphthalenylmethoxy, 2-naphthalenylmethoxy, (4-*tert*-butyl)methoxy, (3I-Ph)CH_2O, (4Br-Ph)O, (2Br-Ph)O, 30   (3Br-Ph)O, (4I-Ph)O, (3Br-Ph)CH_2O, (3,5-Br_2-Ph)CH_2O,
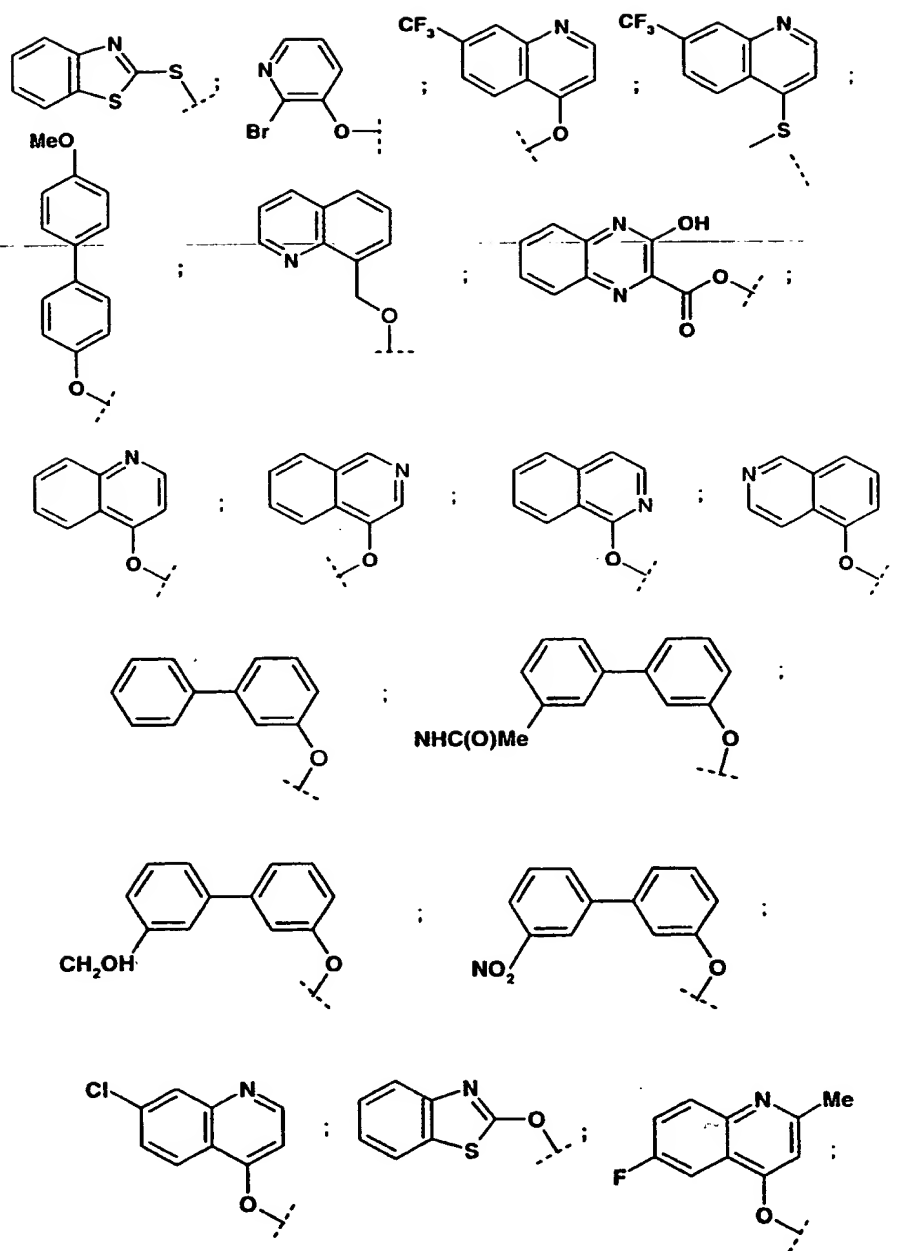
5   28. The compound of formula I according to claim 27, wherein R_{17} is O-Bn, 1-naphthalenylmethoxy, 2-naphthalenylmethoxy,
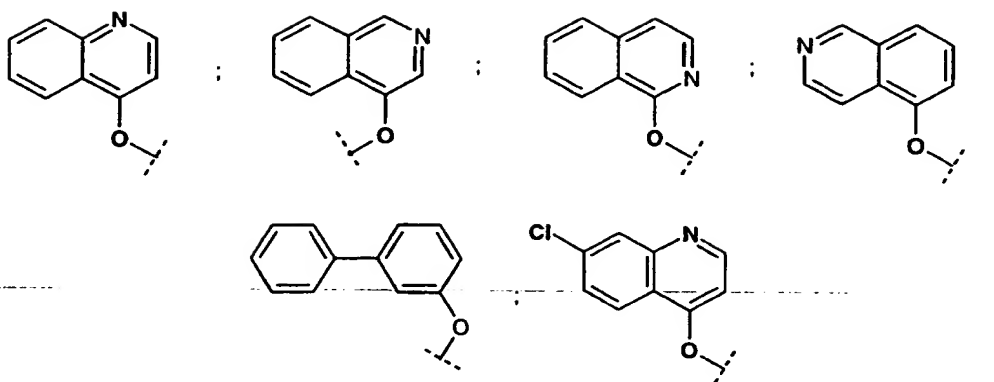
29.   The compound of formula I according to claim 27,
wherein R_{17} is O-Bn, 1-naphthalenylmethoxy, or 2-naphthalenylmethoxy.

30.   The compound of formula I according to claim 1,
wherein **Q** is:
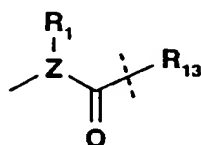
wherein **Z** is preferably CH or N;

when **Z** is CH: R_{13} is H; CF_3; CF_2CF_3; CH_2-R_{14}; C(O)NH-R_{14}, $NR_{14}R_{14'}$ wherein R_{14} and $R_{14'}$ are as defined above
with the proviso that R_{13} is not an α-amino acid or an ester thereof; and

when **Z** is N: R_{13} is phenyl, or C_{7-16} aralkyl,

or   .
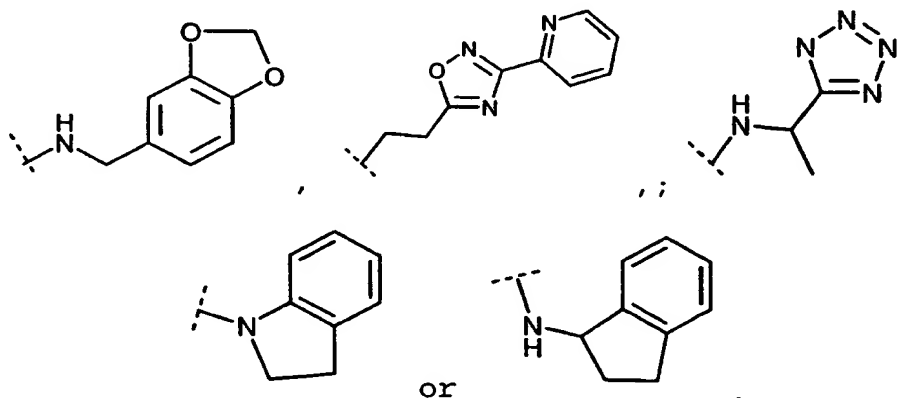
31.   The compound of formula I according to claim 30, wherein when Z is CH: R_{13} is H; NH-R_{14} or C(O)NH-R_{14}; wherein R_{14} is phenyl or C_{7-16} aralkyl.

5   32.   The compound of formula I according to claim 31, wherein R_{13} is H; or C(O)NH-R_{14}; and R_{14} is benzyl or CH(Me)Ph.

33.   The compound of formula I according to claim 30,
10   wherein when Z is N: R_{13} is naphthyl, NH-CH(Me)Ph, NH-CH(Et)Ph,

or
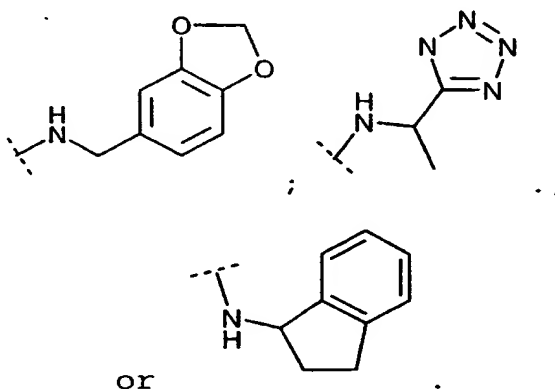
15   34.   The compound of formula I according to claim 33, wherein R_{13} is, NH-CH(Me)Ph, or

or
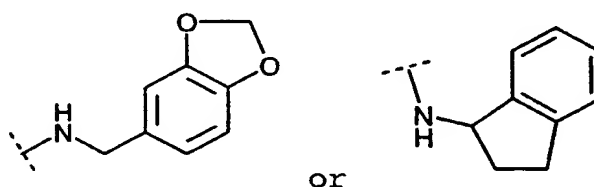
35.   The compound of formula I according to claim 1,
20   wherein Q is a phosphonate group of the formula:
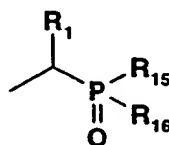
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wherein R_{15} and R_{16} are independently preferably C_{6-12} aryloxy.

36. The compound of formula I according to claim 35, wherein R_{15} and R_{16} are each phenoxy.

37. The compound of formula I according to claim 1, wherein R_1 C_{1-6} alkyl or C_{1-6} alkenyl optionally substituted with halo.

38. The compound of formula I according to claim 37, wherein R_1 is C_{1-5} alkyl or C_{1-4} alkenyl optionally substituted with fluoro.

39. The compound of formula I according to claim 38, wherein R_1 is ethyl, propyl, isopentyl, or allyl.

40. The compound of formula I according to claim 1, wherein **B** is an acyl derivative of formula $R_{11}C(O)-$ wherein R_{11} is: C_{1-6} alkyl optionally substituted with carboxyl, C_{1-6} alkanoyloxy or C_{1-6} alkoxy; C_{3-7} cycloalkyl optionally substituted with carboxyl, MeOC(O), EtOC(O) or BnOC(O); 3-carboxypropionyl (DAD) or 4-carboxybutyryl (DAE); or

HOOCCH₂N⟨ ⟩NCOOBn ;
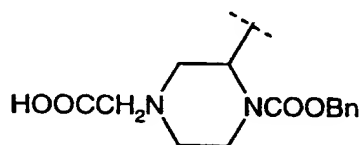
R_6, when present, is the side chain of Asp or Glu;
R_5, when present, is the side chain of an amino acid selected from the group consisting of: D-Asp, L-Asp, D-Glu, L-Glu, D-Val, L-Val, D-*tert*-butylglycine (Tbg), and L-Tbg;
Y is H or C_{1-3} alkyl;

R_4 is the side chain of an amino acid selected from the group consisting of: Val, cyclohexylglycine (Chg), Tbg, Ile, or Leu;

R_3 is the side chain of an amino acid selected from the group consisting of: Ile, Chg, Cha, Val or Glu;

W is:
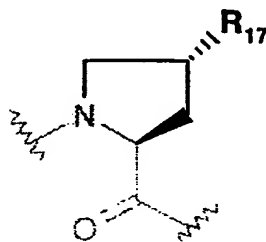
wherein R_{17} is OR_{12} wherein R_{12} is a C_6 or C_{10} aryl or C_{7-16} aralkyl, said first aryl or aralkyl optionally substituted with C_{1-6} alkyl, C_{3-7} cycloalkyl, NH_2, OH, SH, halo, C_{1-6} alkoxy, carboxyl, carboxy(lower)alkyl, or a second aryl or aralkyl; said first and second aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

Q is:
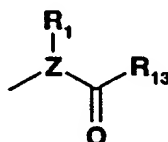
wherein **Z** is N; and R_{13} is phenyl, C_{7-16} aralkyl,

or   ;
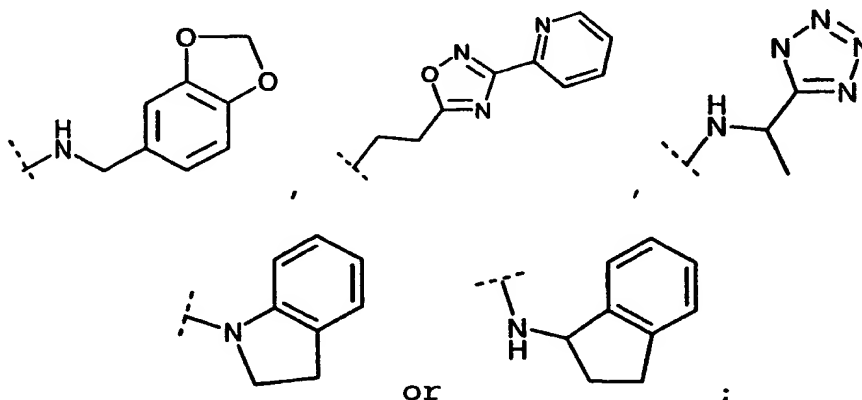
and R_1 is C_{1-6} alkyl or C_{1-6} alkenyl optionally substituted with halo.

41. The compound of formula I according to claim 40, wherein **B** is acetyl, 3-carboxypropionyl (DAD), 4-carboxybutyryl (DAE), $AcOCH_2C(O)$,
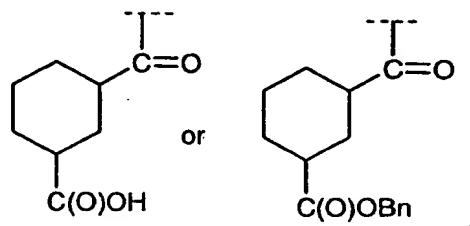
R_6, when present, is the side chain of Asp, or R_6 is absent;

R_5, when present, is the side chain of D-Glu; or R_5 is absent;
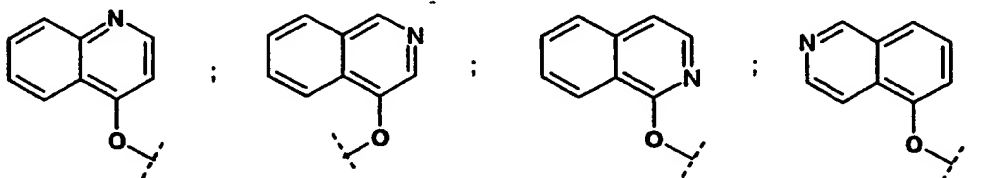
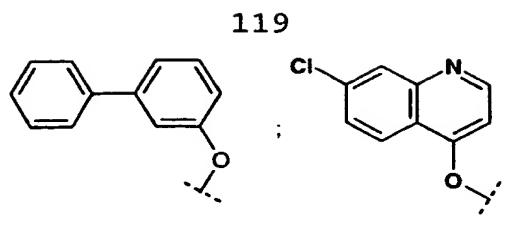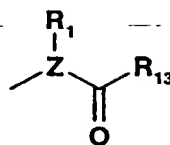R_4 is the side chain of Chg;

R_3 is the side chain of Val;

W is
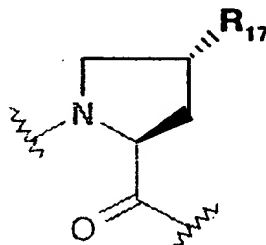
wherein R_{17} is Bn; $PhCH_2CH_2$; $PhCH_2CH_2CH_2$; O-Bn; 1-naphtyloxy; 2-naphtyloxy; 1-naphthalenylmethoxy; 2-naphthalenylmethoxy;

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Q is:

wherein **R₁₃** is, NH-CH(Me)Ph, or

5   or   , and
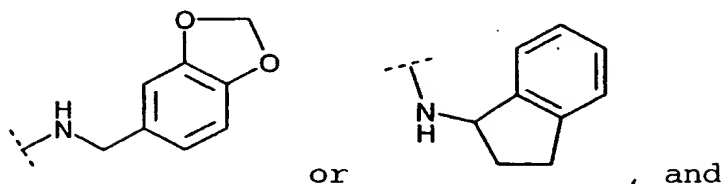
R₁ is ethyl, propyl, isopentyl, or allyl.

42.   The use of a compound according to claim 1 for the treatment of a hepatitis C infection in a mammal
10   comprising administering thereto an anti-hepatitis C virally effective amount of the compound of formula I according to claim 1.

43.   A pharmaceutical composition comprising an anti-
15   hepatitis C virally effective amount of a compound of formula I according to claim 1, or a therapeutically acceptable salt or ester thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.
20

44.   A method of treating a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of the compound of formula I according to claim 1, or a
25   therapeutically acceptable salt or ester thereof.

45. A method of inhibiting the replication of hepatitis C virus by exposing the virus to a hepatitis C viral NS3 protease inhibiting amount of the compound of formula I according to claim 1, or a

5   therapeutically acceptable salt or ester thereof.

46. A method of treating a hepatitis C viral infection in a mammal by administering thereto an anti-hepatitis C virally effective amount of a

10   combination of the compound of formula I according to claim 1, or a therapeutically acceptable salt or ester thereof.

47. A pharmaceutical composition according to claim

15   43, comprising an additional immunomodulatory agent selected from the group consisting of: α-, β-, and δ-interferon.

48. A pharmaceutical composition according to claim

20   43, comprising an additional antiviral agent selected from the group consisting of: ribavirin and amantadine.
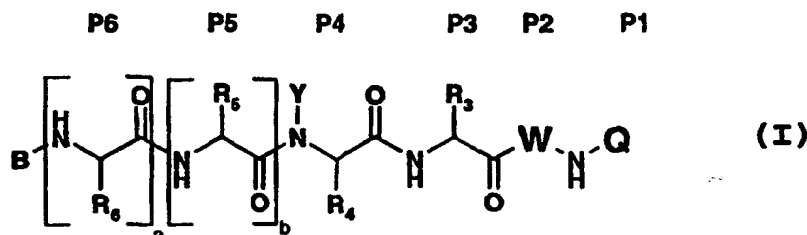
49. A pharmaceutical composition according to claim

25   43, additionally comprising other inhibitors of HCV protease.

(51) International Patent Classification 6 : C07K 14/18, 5/107, 5/093, A61K 38/16, 38/04	A3	(11) International Publication Number: **WO 99/07734** (43) International Publication Date: 18 February 1999 (18.02.99)

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(71) Applicant *(for all designated States except US)*: BOEHRINGER INGELHEIM (CANADA) LTD. [CA/CA]; 2100 Cunard, Laval, Québec H7S 2G5 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LLINAS-BRUNET, Montse [CA/CA]; 10543 Bélair, Pierrefonds, Québec H2V 2W8 (CA). BAILEY, Murray, Douglas [CA/CA]; 344 Groulx, Pierrefonds, Québec H8Y 1B3 (CA). HALMOS, Teddy [CA/CA]; 1935 Jean Ricard #8, Laval, Québec H7T 2K4 (CA). POUPART, Marc-André [CA/CA]; 101 Aimé Séguin, Laval, Vimont, Québec H7M 1B3 (CA). TSANTRIZOS, Youla [-/-]; 1590 Champigny, Saint-Laurent, Québec H4L 4P7 (CA).

(74) Agent: VAN ZANT, Joan, M.; Van Zant & Associates, Suite 1407, 77 Bloor Street West, Toronto, Ontario M5S 1M2 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
20 May 1999 (20.05.99)

(54) Title: HEPATITIS C INHIBITOR PEPTIDE ANALOGUES

(57) **Abstract**

Compound of formula (I) active against the Hepatitis C virus, wherein B is an acyl derivative; a is 0 or 1; R_6, when present, is carboxy(lower)alkyl; b is 0 or 1; R_5, when present, is C_{1-6} alkyl, or carboxy(lower)alkyl; Y is H or C_{1-6} alkyl; R_4 is C_{1-10} alkyl; R_3 is C_{1-10} alkyl; W is $-NH-CH(R_2)-C(O)-$, wherein R_2 is C_{1-6} alkyl; C_6 or C_{10} aryl; C_{7-16} aralkyl; or carboxy(lower)alkyl; or W is a proline derivative; Q is a group of the formula $-Z(R_1)-C(O)-R_{13}$, wherein Z is CH or N; R_1 is C_{1-6} alkyl or C_{1-6} alkenyl both optionally substituted with thio or halo; and R_{13} is an activated carbonyl substituent, or Q is a phosphonate group of the formula $-CH(R_1)-P(O)R_{15}R_{16}$ wherein R_{15} and R_{16} are independently C_{6-20} aryloxy; and R_1 is as defined above.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C07K14/18   C07K5/107   C07K5/093   A61K38/16   A61K38/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C07K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORI E.A.: "The N-terminal region of NS3 serine protease of HCV is important to maintain its enzymatic integrity" BIOCHEM.BIOPHYS.RES COMM., vol. 231, no. 3, 24 February 1997, pages 738-742, XP002086571 see page 740, column 2 ---	1,2, 4-12, 15-17, 19-21, 24,30, 31,37, 42-49
P,X	WO 98 17679 A (DEININGER DAVID D ;MURCKO MARK A (US); VERTEX PHARMA (US); FARMER) 30 April 1998 cited in the application see the whole document --- -/--	1-49

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 March 1999	01/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office. P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

page 1 of 2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 22496 A (HOFFMANN LA ROCHE) 28 May 1998 see the whole document ---	1-26, 30-32, 37-39, 42-49
A	LANDRO E.A.: "Mechanistic role of NS4A peptide cofactor with the truncated NS3 protease of HCV: elucidation of the NS4A stimulatory efect via kinetic mapping and inhibitor mapping" BIOCHEMISTRY, vol. 36, no. 31, 5 August 1997, pages 9340-9348, XP002086573 see the whole document -----	1-49

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INTERNATIONAL SEARCH REPORT

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   Remark:- Although claims 42 and 44-46 are directed to a method of treatment
   of the human/animal body, the search has been carried out and
   based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:   1-39,42-49 partially
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out, specifically:

   see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all
   searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report
   covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

Claims Nos.:   1-39,42-49(all partially)

The claims are unduly broad and speculative, allowing substitutions in every position and lacking any significant constant structural entity. Therefore a meaningful and economically feasible search could not encompass the complete subject-matter of the claims. Consequently the search has been directed to the exemplified compounds and closely related analogs (and their use) and extended to compounds having the same activity and has been complete for the subject-matter of the claims 40 and 41 (Art.17(2)(a)(ii) and (b) PCT, PCT Guidelines CIII,2.1 and CIII,3,7).

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9817679	A	30-04-1998	AU	5147798 A	15-05-1998
WO 9822496	A	28-05-1998	AU	5551098 A	10-06-1998
			HR	970618 A	31-08-1998
			US	5866684 A	02-02-1999